EFFECTS OF AGING NEW CROP WHEAT AND WHOLE WHEAT FLOUR ON BREADMAKING QUALITY AND GLYCOLIPID COMPOSITION

by

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Abstract

Whole wheat flour has become a popular ingredient in baked goods. Consumers are seeking the added benefits of consuming healthy phytochemicals and bioactive compounds. As the demand for wholemeal flour increases it is important to understand the factors contributing to changes in whole wheat flour breadmaking quality as a function of both wheat and flour age. In wholemeal flour, the lipid composition appears to be a factor causing variable baking quality. Changes that occur in both freshly harvested wheat and milled flour can cause variations in baking quality. Bakers' attempts to adjust formulations and processes are often unsuccessful. The objective of this study was to determine the effects of aging both new crop wheat and freshly milled flour at 2 different temperatures on baking quality and glycolipid composition. This study aged freshly harvested Overley hard red winter wheat at RT (23˚C) and FZ (-26˚C) for 8, 50, and 91 days before milling. Whole wheat flour baking and lipid extraction studies were performed incrementally over 31 days of flour storage after each milling. Glycolipid structure and amounts were measured using automated electrospray ionization-tandem mass spectrometry. The glycolipids analyzed in this study were DGDG (digalactosyldiglycerol), MGDG (monogalactosylmonoglycerol), MGMG (monogalactosylmonoglycerol), and DGMG (digalactosylmonoglycerol). Both the wheat and flour stored at -26˚C produced larger mean loaf volumes than did the 23˚C treatment for all 3 wheat ages. The FZ and RT DGDG:MGDG ratios were similar over both wheat and flour age so changes to the glycolipid fraction did not appear to be the cause of the significant difference between volumes of bread baked from samples stored at RT and FZ. There was limited change in mean volume over flour age except for an increase at the FZ 91 day point. The DGDG:MGDG ratios were generally unchanged over flour age. Loaf volumes were the same over wheat age except for an unexpected drop during the 50 day wheat study. The corresponding DGDG:MGDG ratios did not show any significant differences over wheat age. The FZ storage temperature might be inhibiting some biochemical change not affecting the glycolipids, leading to higher volumes than the RT storage treatment.

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Dedication

To my parents Christy and Leland Mense and my siblings Stephanie, Steven and Carolyn Mense.

Chapter 1 - Introduction

A food product is considered to be whole grain if the proportions of the individual components within the kernel are present in the same proportions in the final product (Slavin 2004). Whole grains are known to be good sources for vitamins, minerals and phytochemicals with antioxidant activity (Slavin 2004; Liu 2007). Phytochemicals such as lignin, B-glucan, Vitamins E and B, and inulin are present in the wheat kernel (Liu 2007). These compounds are primarily found in the bran, germ and aleurone (Liu 2007) and can reduce the chance of contracting some chronic diseases (Liu 2007). Additionally bioactive compounds present in whole grain such as fermentable carbohydrates, phytic acid, phytoestrogens and phytosterols are associated with claims that whole grain consumption reduces the risk for colon cancer, heart disease and diabetes (Slavin 2004; Newby et al 2007). Whole grain wheat also contains unsaturated lipids which lower LDL cholesterol (Slavin 2004). Those include oleic, linoleic and linolenic acid along with palmitate and are present at 2g per 100g of whole wheat (Slavin 2004).

The 2005 USDA Dietary Guidelines recommended that half of the grain servings consumed daily should come from whole grains (USDA 2005). With more evidence supporting the value of consuming whole grains, North American consumers want bread with the added benefits of consuming dietary fiber, protein, antioxidants, vitamins, and minerals (Dewettinck et al 2008). Thus, as consumers become more health conscious, the incorporation of whole grains into bread has become very popular in the baking industry (Dewettinck et al 2008). Mintel (2012) reported that worldwide, 36.4% of new products launched in 2011 that made whole grain claims were bakery products and the amount of whole grain claims made on products worldwide has increased by 1960% between 2000 and 2011. Because whole wheat flour production in the U.S. has tripled between 2002 and 2011 (Doblado-Maldonado et al 2012), determining factors contributing to whole wheat flour changes with increased storage time is crucial (Barnes and Lowy 1986).

Changes that occur in freshly harvested wheat can cause variations in the baking quality of milled flour (Shelke et al 1992a). Consequently, bakers have to adjust formulations and production processes in an attempt to correct the problem but their attempts are, generally, unsuccessful (Shelke et al 1992a). The causes of quality inconsistencies found when baking

bread using flour from new crop wheat have not yet been verified (Shelke et al 1992a). Changes occurring in both the new crop wheat and the milled flour should be identified, measured, and understood so efforts can be made to limit the variations that bakers face (Shelke et al 1992a). These variations should be studied as a function of both wheat and flour age. Research focused on the effects of wheat and flour storage on baking quality and lipid composition could, therefore, help manufacturers produce more consistent products.

Storing freshly harvested wheat (hard and soft) over time is known to improve baking quality when using refined flour (Shellenberger 1939; Posner and Deyoe 1986; Shelke et al 1992a). Studies have also found that freshly milled refined hard and soft wheat flour showed an improvement in baking quality over flour age (Posner and Deyoe 1986; Shelke et al 1992a; Chen and Schofield 1996). Unlike refined flour, whole wheat flour baking quality is believed to decrease over time (Tait and Galliard 1988).

Wholemeal flour contains, in addition to the starchy endosperm, the bran and germ components. Whole wheat milling increases the amount of neutral lipids compared to polar lipids in flour due to the germ and aleurone layers containing higher percentages of neutral lipids (Larsen et al 1989). When stored together, the bran and germ have a synergistic effect on stability due to the release of lipases which hydrolyze fatty acids into free fatty acids (Galliard 1986a; Galliard 1986b). The germ releases lipoxygenase which reacts with those free fatty acids to start the process of oxidative degradation (Chung et al 1978; Galliard 1986a; Galliard 1986b). Because the whole wheat kernel contains more unsaturated fatty acids, lipid degradation in whole wheat flour is likely. Change in the lipid composition appears to play a role is reducing the baking quality of wholemeal flour (Galliard 1986b).

Glycolipids have been shown to have the greatest improving effect on flour baking quality (Prieto et al 1992). Their optimal HLB value and high polarity may allow them to stabilize gas cells at the dough liquor-gas cell interface especially during proofing and oven spring (Selmair and Koehler 2009). Hoseney et al (1970) found that the simultaneous bonding between free MGDG and DGDG and gliadin and glutenin restored carbon dioxide retention of the lipoprotein complex and improved bread loaf volume. Detrimental changes to the glycolipid fraction during storage could affect bread quality. How the glycolipid fraction changes in new crop wheat and freshly milled whole wheat flour over time is unknown.

This research intends to document the changes and determine the mechanism that causes the changes in whole wheat flour breadmaking quality over time from new crop wheat. It will attempt to identify and measure changes in the flour's glycolipid fraction and breadmaking quality of new crop wheat by aging the wheat kernel and whole wheat flour for increasing periods of time at 2 different temperatures. Freshly milled flour may be expected to improve in the short term but that, overtime, baking quality would decrease. The mechanism that determines the change in baking quality over time has not been found (Warwick et al 1979).

Objectives

1. Determine if and how postmilling storage time and temperature affect glycolipid composition and breadmaking quality of whole wheat flour.
2. Determine the effects of aging new crop hard wheat at two different temperatures on glycolipid composition and baking quality

Hypotheses

Wheat and whole wheat flour will change over time

- Room temperature conditions will increase the rate of change
- Freezing conditions will slow down change

Changes in the glycolipid composition will occur over wheat and whole wheat flour age

- This will occur at a faster rate at room temperature
- It will be reflected in changed baking quality

Chapter 2 - Literature Review

2.1 Lipid Classification

Lipids are defined as "fatty acids and their derivatives and substances related biosynthetically or functionally to these compounds (American Oil Chemists Society (AOCS) online Lipid Library (http://lipidlibrary.aocs.org/)). This section first will classify lipids by biochemistry, then extraction methods, and lastly by their kernel location (Chung et al 2009). (Appendix A: lipid abbreviations)

2.11 Biochemistry

Lipids are divided into simple and complex categories (Pyler 1988; Chung et al 2009; Pareyt et al 2011). Simple lipids (e.g. triacylglycerols (TAG), diacylglycerols (DAG), and monoacylglycerols (MAG), fatty acids (FA), and sterols) are nonpolar and can be broken down into 2 structures (Fahy et al 2005; Chung et al 2009; Pareyt et al 2011). The 2 structures could consist of free fatty acids (FFA) and acylglycerols or monohydric alcohols and FFA. Complex lipids such as glycolipids and phospholipids are polar and can be hydrolyzed into 3 or more chemical compounds which consist of their acylglycerols and polar moiety (Fahy et al 2005; Chung et al 2009; Pareyt et al 2011).

Fatty acids contain reactive polar carboxylic acids with long hydrophobic chains composed of methylene groups (Fahy et al 2005; Chung et al 2009). The carboxylic acid can react with the hydroxy group of glycerol via a condensation reaction to form TAG, DAG and MAG (Pyler 1988). Triacylglycerols are formed when all 3 hydroxyl groups of glycerol have ester bonds linking them to fatty acids (Pyler 1988; Chung et al 2009). Triacylglycerols account for ~20.8% of refined wheat flour lipids while DAG and MAG contribute ~12.2% and ~1.3% respectively (Macmurray and Morrison 1970). FFA make up 7% of wheat flour lipids (Macmurray and Morrison 1970). Polyunsaturated fatty acids contain 2 or more double bonds while monounsaturated fatty acids have 1 double bond (Pareyt et al 2011). The most common polyunsaturated FA in wheat are linoleic (18:2), and linolenic acids (18:3) (Chung et al 2009). Linoleic acid is the most prevalent FA making up 55-60% of all FA in wheat (Chung et al 2009). Oleic acid (18:1) is a common monounsaturated fatty acid found in wheat (Chung et al 2009). Palmitic (16:0) and stearic (18:0) fatty acids have no double bonds and all of their carbons have a full complement of hydrogens (Pyler 1988). Unsaturated hydrocarbon chains can be found as cis (Z) or trans (E) conformations (Pareyt et al 2011). The trans conformation orients hydrogens so that they are on opposite sides of the double bond while cis hydrogens are on the same side (Chung et al 2009).

Glycolipids contain 1 or more monosaccharide residues attached to a hydrophobic moiety by a glycosidic linkage (IUPAC-IUB Joint Commission on Biochemical Nomenclature 1997). The hydrophobic groups may consist of acyl glycerols, ceramides, sphingoids, and prenyl phosphates (IUPAC-IUB Joint Commission on Biochemical Nomenclature 1998). Glycolipids are most commonly found in the endosperm with 30.7 - 38.3% as nonstarch lipids and 1.2-5.5%

in the starch lipids (Chung et al 2009). Their amphiphilic nature allows them to interact at gas-liquid (Sroan and MacRichie 2009b) and aqueous-liquid interfaces (Law 1960). Glycolipids have been shown to have the greatest improving effect on flour baking quality (Prieto et al 1992). The 18:2/18:2 and 16:0/18:2 fatty acid combinations are most common for MGDG and DGDG glycolipids (Prieto et al 1992). The DGDG is the most common glycolipid found in flour followed by MGDG (Chung et al 2009). Common glycolipids in flour are acylated steryl glycosides (ASG) (Chung et al 2009), monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), digalactosylmonoglycerol (DGMG), and monogalactosylmonoglycerol (MGMG) (Finnie et al 2009).

Phospholipids in their basic form are called phosphatidic acids (Chung et al 2009). Two fatty acids are bound by ester linkages to 2 hydroxyl groups of glycerol and a phosphate group is bound to carbon 3 (Chung et al 2009). Phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) are the most common phospholipids present in wheat flour (Chung et al 2009).

2.12 Extraction Methods

As mentioned above, wheat lipids can also be categorized by extraction methods (Chung et al 2009). Lipids within the endosperm can be characterized based on how they are bound within the endosperm matrix and what solvents they are soluble in (Finnie et al 2009). Total flour lipids include those bound to starch granules while non-starch lipids do not (Finnie et al 2009).

Once nonstarch flour lipids are extracted they can be further categorized into free and bound lipids (Carr et al 1992; Finnie et al 2009; Pareyt et al 2011). Extraction of nonstarch lipids can be performed at ambient temperature without any disruption of the starch (Finnie et al 2009). Free lipids are those extracted with petroleum-ether or hexane solvents and are immiscible in water (Daftary et al 1968; Chung et al 2009). Free lipids are soluble in nonpolar solvents and so are classified as nonpolar lipids (Finnie et al 2009). The free lipids account for 35% of the total wheat flour lipids (Pareyt et al 2011). The free lipid flour extraction contains 26% nonpolar lipids such as TAG, DAG, MAG, and FFA (Pareyt et al 2011). The other 9% contains polar lipids such as DGDG, MGDG, NAPE (N-lysophosphatidylcholine) and PC (Pareyt et al 2011). Free polar lipids have higher amounts of monogalactosyl glycerides (Daftary et al 1968).

After free lipid extraction the bound lipids are extracted from flour with a polar solvent such as water-saturated butanol or propan-2-ol (Daftary et al 1968; Greenblatt et al 1995). Bound lipids are soluble in polar solvents so they are classified as polar lipids (Finnie et al 2009). The bound lipids constitute 25% of the total wheat flour lipids. Bound lipids are 100% polar and composed of glycolipids and phospholipids (Pareyt et al 2011). Polar bound lipids are mainly composed of digalactosyl glycerides (Daftary et al 1968). The bound polar lipids also contain phosphatidyl choline, phosphatidyl serine, and phosphatidyl ethanolamine (Daftary et al 1968).

The internal starch lipids, as the name implies, exist internally within starch granules and can occur as an amylose-lipid complex (Carr et al 1992). The nonstarch lipids account for 60% of the total wheat flour lipids and internal starch lipids 40%, of that total share (Pareyt et al 2011). Lysophosphatidylcholine is found primarily associated with amylose (Carr et al 1992). Polar solvents such as water saturated butanol, which can be heated to 90˚C (gelatinizing the starch) aide in the extraction of internal starch lipids (Chung et al 2009, Finnie et al 2009). The internal starch lipids are all polar (Pareyt et al 2011). Phospholipids such as LPC, LPG (lysophosphatidylglycerol), and LPE (lysophosphatidylethanolamine) are the main classes present as internal starch lipids (Pareyt et al 2011).

2.13 Physical Location

Lipids can also be described by their physical location. In total, lipids make up 2.5-3.3% of the wheat kernel (Finnie et al 2009). The germ and aleurone contain 26-29% and 24-31% of that total lipid, respectively (Chung et al 2009). Wheat lipids are also found in the endosperm as nonstarch lipids (28-31%) and starch associated lipids (15-21%) (Chung et al 2009).

The aleurone and germ contain primarily nonpolar lipids (Finnie et al 2009). The nonpolar lipids in the wheat kernel are TAG, DAG, MAG, free sterols, FFA, and sterol esters (Finnie et al 2009). Germ lipids are mostly nonpolar (83.7 to 84.8%) with TAG being the most prevalent form (Chung et al 2009). Polar lipids such as glycolipids (0-3.6%) and phospholipids (15.2-16.8%) are present at low levels in the germ (Chung et al 2009). The aleurone has nonpolar (72.3-79.5%), glycolipid (6.7-9.8%) and phospholipid (13.8-17.9%) contents similar to those found in the germ (Chung et al 2009; Finnie et al 2009). The nonstarch lipids have substantially more glycolipids (30.7-38.3%) while the starch lipids have less (1.2-5.5%) (Chung et al 2009). Starch lipids are composed of 90.1-94.4% phospholipids while nonstarch lipids

contain 23.6-34.4% (Chung et al 2009). The endosperm contains the majority of the polar lipids such as glycolipids and phospholipids (Finnie et al 2009).

2.2 Whole Wheat and Refined Flour Lipid Composition

Bekes et al (1986) analyzed the nonstarch lipid composition of whole wheat flour and refined flour. They analyzed 25 wheat varieties of whole wheat flour and 26 of refined flour from wheat grown in the same location. The average wheat kernel is composed of 2.32% nonstarch lipids while straight grade flour contains roughly 1.55% (Pyler 1988). Bekes et al (1986) found that whole wheat flour from the same varieties contained 3006 mg/100g nonstarch lipids while refined flour averaged 1786 mg/100g (Bekes et al 1986).

Bekes then fractionated whole wheat flour nonstarch lipids into free and bound lipids. They were found to be present at 2084 mg/100g (69.33%) and 921 mg/100g (30.64%) respectively (Bekes et al 1986). In contrast, the wheat kernel nonstarch lipids were composed of 68.10% free lipids and 31.90% bound lipids (Pyler 1988). Refined flour free and bound lipids were reported at 1086 mg/100g (60.81%) and 705 mg/100g (39.47%) respectively (Bekes et al 1986). Similarly Pyler (1988) reported that refined flour free lipids make up 61.29% of the nonstarch lipids and bound lipids 39.35%. Refined flour contains a higher percentage of polar bound lipids and fewer nonpolar lipids than does whole wheat flour.

The same study fractionated free lipids into neutral lipids, glycolipids, and phospholipids (Bekes et al 1986). The whole wheat flour contained 1922 mg/100g neutral lipids, 60 mg/100g glycolipids, and phospholipids averaged 98 mg/100g (Bekes et al 1986). Refined flour neutral lipids were reported at 946 mg/100g, 69 mg/100g glycolipids and 68 mg/100g phospholipids (Bekes et al 1986). Whole wheat flour contains more phospholipids and neutral lipids while refined flour has more glycolipids in the free lipid fraction.

Wheat kernels contain 44.4-56.9% neutral lipids that are composed of mostly triacylglycerols which make up 29.5-47.2% of the wheat lipids (Chung et al 2009). Milled flour contains fewer neutral lipids at 42.1-43.2% and is also made up, primarily, of triacylglycerols (Chung et al 2009). Wheat kernels have fewer glycolipids (12.4-14.4%) than milled flour (16.4-19.0) (Chung et al 2009). Phospholipid levels are similar for milled flour and wheat at 37.8-39.0% and 30.6-41.5% respectively (Chung et al 2009).

Finnie et al (2009) reported that refined flour for the HRW cultivar of Overley contains slightly more total polar lipids (4762 nmol of lipid/g of sample) than did whole meal flour (4073 nmol/g). Total polar lipids in this study were determined by totaling the bound and free phospholipid and glycolipid fractions (Finnie et al 2009). Whole meal flour contained more total phospholipids (1585 nmol/g) than did refined flour (713 nmol/g) (Finnie et al 2009), while white flour contained more glycolipids (4049 nmol/g) than did whole meal flour (2488 nmol/g) (Finnie et al 2009). The most common polar lipids in the free and bound fractions of both refined and whole meal flour were DGDG, MGDG, and PC (Finnie et al 2009). DGDG was the most prevalent glycolipid in both wheat (5.4-9.5%) and flour (8.7-11.7%) (Chung et al 2009). The DGDG and MGDG 36:4 lipids were the glycolipid classes most prevalent in wholemeal and refined flours (Finnie et al 2009). Both classes were more heavily concentrated in the bound fraction. For wholemeal flour, the DGDG and MGDG lipids constituted 30.83 mol% and 29.43 mol% of the total polar lipids, respectively (Finnie et al 2009). The refined flour DGDG and MGDG lipids constituted 50.47 mol% and 32.37 mol% of the total polar lipids, respectively (Finnie et al 2009). For wholemeal and refined flour, PC lipids consisted of 22.92 mol% and 8.96 mol% of the total polar lipids, respectively (Finnie et al 2009).

Inkpen et al (1969) found that hard wheat and refined hard wheat flour fatty acids were 79.8% and 76.98% unsaturated respectively. Similarly, Pyler (1988) reported that the wheat kernel fatty acids were 81.66% unsaturated and refined flour fatty acids were 78.85% unsaturated. Linoleic acid was the most prevalent fatty acid in both the whole wheat kernel and refined flour (Pyler 1988), comprising 62.13% of total fatty acids in the kernel and 62.5% in refined flour (Pyler 1988). Because the whole wheat kernel contains more unsaturated fatty acids, lipid degradation in whole wheat flour is likely and could be the cause of a loss in baking quality over time.

2.3 Flour Lipids in Baking

2.31 Polar and Nonpolar lipid in Baking

Polar lipids are known to improve baking quality and reduce crumb firming while neutral lipids have negative effects on loaf volume and produce a hard crumb (Pomeranz et al 1965;

Ponte and DeStefanis 1969; Larsen et al 1989). The addition of nonpolar lipids to flour has been found to decrease loaf volume (MacRitchie and Gras 1973; DeStefanis and Ponte 1976). Chung et al (1980) reported that as the nonpolar/polar lipid ratio decreased loaf volume increased for HRW flour.

Glycolipids and phospholipids have both polar and nonpolar characteristics (Selmair and Koehler 2009). Although they both have surface active properties which allow them to function as emulsifiers in the baking process (Selmair and Koehler 2009) glycolipids were found to improve baking quality more than did phospholipids (Daftary et al 1968).

Selmair and Koehler (2009) purified glycolipids from various lecithin sources and compared their baking quality with well known emulsifiers (DATEM, SSL, monoacylglycerides) and synthetic polar lipids. Although all the glycolipids improved loaf volumes, the DGDG lipid class showed the largest overall loaf volume increase at an incorporation level of 0.6% in a 10g bake test (Selmair and Koehler 2009). The nonpolar lipids were detrimental to loaf volume at increasing concentrations (Selmair and Koehler 2009).

The DGDG were the most polar of the glycolipids tested with a HLB between 9.9-10.5 (Selmair and Koehler 2009). Their optimal hydrophilic lipophilic balance and high polarity may allow them to stabilize gas cells at the dough liquor/gas cell interface especially during proofing and oven spring (Selmair and Koehler 2009). The less polar glycolipids, may allow for more native lipids to interact at the interface enhancing gas cell stability (Selmair and Koehler 2009). Sroan et al (2009a) also concluded that whole flour lipids (polar plus nonpolar lipid) have surface active properties along the gas/liquid interface that allow them to stabilize liquid lamellae surrounding the cells during proofing and oven spring found on the inner side of the gluten-starch matrix. Polar lipids remain viscous at 100˚C, nonpolar lipids are liquid at approximately 25˚C, and total unfractionated lipids melted at 40˚C (Pomeranz et al 1966). The higher melting point of polar lipids could (potentially) aid in gas retention during the baking process (Pomeranz et al 1966).

Lipids at the gas/liquid interface can form detrimental expanded monolayers or form condensed monolayers that enhance loaf volume (Sroan et al 2009b). Polar groups such as those of DGDG and saturated fatty acids can form condensed monolayers which add elastic stability to gas cells (Sroan et al 2009b). Nonpolar lipids and long chain unsaturated lipids are expected to form expanded monolayers that can be compressed. This would decrease loaf volume (Sroan et

al 2009b). These assumptions were confirmed when polar and nonpolar lipids were found to increase and decrease loaf volume respectively while the long chain saturated fatty acids had no effect (Sroan et al 2009b). DeStefanis and Ponte (1976) also found that the addition of unsaturated linoleic fatty acids lowered loaf volumes more than did saturated palmitic acid especially at high levels (DeStefanis and Ponte 1976).

Flour lipids could have their effect by stabilizing gas cells within dough (MacRitchie 1981). In fact, lipids and protein may work together to stabilize gas cells at the gas/liquid interface (MacRitchie 1981). In this context, protein quality is known to affect the degree to which lipids improve loaf volume (MacRitchie 1981). If a low quality protein is substituted into defatted flour, subsequent lipid additions do not improve loaf volumes to the same degree as if high quality protein was present (MacRitchie 1981). The molecular weight distribution of polymeric proteins has to allow adequate gas expansion through dough extensibility and provide strength due to entanglement (Sroan et al 2009a).

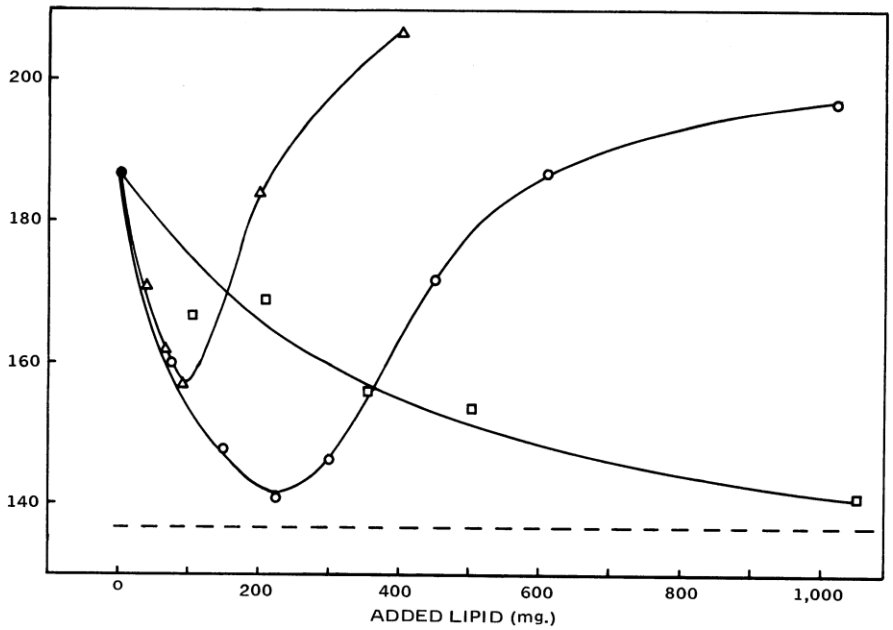
Glycolipids can form hydrophilic hydrogen bonds and hydrophobic bonds with gliadin and glutenin respectively (Chung et al 1978; Hoseney et al 1970; Wehrli and Pomeranz 1970). The glycolipid's carbohydrate group can provide a stable structure for dough through hydrogen bonding with gliadin's polar amino acid groups and starch (Wehrli and Pomeranz 1970). Hydrophobic bonds between glutenin and glycolipids are most common in water but hydrogen bonding can also occur (Wehrli and Pomeranz 1970). The phospholipids interact with gliadin too which helps stabilize gas cells in dough (Pareyt et al 2011). Polar and some nonpolar lipids become bound to the gluten proteins during mixing which may assist in their stabilization of gas cells (Pareyt et al 2011). Hoseney et al (1970) found that the simultaneous bonding between free MGDG and DGDG and gliadin and glutenin restored carbon dioxide retention of the lipoprotein complex and improved bread loaf volume. Therefore detrimental changes to the glycolipid fraction during storage could affect bread quality.

2.32 Reconstitution Studies

The addition of polar and whole lipids (polar plus nonpolar lipid) back into defatted flour at low levels decreased loaf volume to a point but additional lipid content caused an increase in loaf volume (cc) as per Figure 2.1 (dough size ~ 50g) (MacRitchie and Gras 1973). The addition of nonpolar lipids decreased loaf volume at all levels as seen in Figure 2.1 (MacRitchie and Gras

1973). Sroan and MacRitchie (2009b) also found that adding back small amounts of free polar and whole (polar plus nonpolar lipid) lipids initially decreased loaf volume but once 30-50% of the whole lipid amount was added back into the defatted flour loaf volume increased dramatically. The cause of this specific response has not been determined (MacRitchie 1981). The positive effects of adding more lipids diminish past a certain point (Pomeranz et al 1965) and adding more than the original amount of lipids back into a bread formula had no effect on loaf volume (Pomeranz et al 1968). When half of the extracted free lipids were added back to defatted but shortening supplemented flour, loaf volumes were fully recovered (Pomeranz et al 1968).

Figure 2.1 Loaf Volume as a Function of Lipid Content.



Change in loaf volume (cc) with addition of lipid back into defatted flour. triangle, polar lipid; circle, whole lipid; square, nonpolar lipid; The dashed line is the volume of the dough at the end of proofing (From MacRitchie, F. and Gras, P.W., Cereal Chem., 50, 292, 1973)

DeStefanis and Ponte (1976) studied the effects of adding nonpolar lipids to defatted flour, intact flour and intact flour with 3% lard. The addition of nonpolar lipids decreased the loaf volume of intact flour containing 3% or 0% lard. Conversely loaf volume increased when nonpolar lipids were added to defatted flour with 0% lard (DeStefanis and Ponte 1976). The addition of free fatty acids decreased the loaf volume of defatted and intact flours but that

detrimental effect was masked when 3% lard was present (DeStefanis and Ponte 1976). Triacylglycerol presence increased the loaf volume of all 3 flours (DeStefanis and Ponte 1976).

In a similar study Ponte and DeStefanis (1969) added nonstarch lipids, polar and/or nonpolar fractions to a formula containing 0% or 3% lard. The nonstarch lipids decreased loaf volume when 3% lard was used but the 0% lard formulation was relatively unaffected (Ponte and DeStefanis 1969). As expected the nonpolar lipids decreased loaf volume while polar lipids increased loaf volume for both formulas (Ponte and DeStefanis 1969).

Chung et al (1980) found that shortening improved the loaf volume of unextracted flour (flour containing natural lipid amounts) but was detrimental to the loaf volume of defatted flours. In this study, flour was defatted then reconstituted with polar lipids which were then removed incrementally from the defatted flour to monitor changes in loaf volume (Chung et al 1980). Without shortening, loaf volume of micro loaves (10g flour) decreased from 84 cc to around 69 cc when 0.23% polar lipid was removed but then increased to 78 cc as more polar lipid (up to 0.64%) was removed (Chung et al 1980). With shortening present the minimum loaf volume ~62cc occurred with the removal of 0.49% polar lipids. This removal of polar lipids had a significant effect on loaf volume (Chung et al 1980) and shortening delayed the negative effects of removing polar lipids.

Pomeranz et al (1968) also found that adding 3% shortening increased loaf volumes and improved crumb grain of unextracted lipid containing flours. Conversely crumb grain worsened when shortening was added to defatted flours (Pomeranz et al 1968). Shortening added to extracted flours reconstituted with nonpolar lipids did not have any negative effects on crumb grain and bread loaf volume (Daftary et al 1968). Pomeranz et al (1965) reported that supplementing unextracted flour with 0.5g polar lipids increased loaf volumes to similar levels as adding 3% shortening (Pomeranz et al 1966). Nonpolar lipids resulted in very little loaf volume improvement (Pomeranz et al 1965). Pomeranz et al (1969) found that adding 0.5g free polar lipids to a pup loaf baking formula improved loaf volume significantly more than did adding 3g shortening, 0.5g lecithin, or sucroesters at 0.5g. Hoseney et al (1972) also experimented with synthetic polar lipids. They found that sucrose monotallowate, sucrose monopalmitate, and sodium and calcium stearoyl-2-lactylates (with shortening present) could replace 0.8% free lipids when removed from flour. With 1.0% additions of the previously mentioned synthetic lipids,

loaf volumes of the original unextracted flour (with shortening) were achieved or exceeded (Hoseney et al 1972).

Two different flours, 1 without free lipids and a premix with 0.08% mostly polar lipids, both with added 3% shortening were studied (Hoseney et al 1969). The individual additions of 0.2% bound polar lipids and 0.6% nonpolar lipids to the petroleum ether defatted flour had no effect on the loaf volume (Hoseney et al 1969). Loaf volume was improved when 0.3% free polar lipids interacted with the 0.6% bound lipids still present in the petroleum ether extracted flour (Hoseney et al 1969). Comparatively when 0.3% free lipids were added to the premix flour without the bound lipids loaf volume decreased (Hoseney et al 1969). The unextracted bound lipids seemed to have an impact on loaf volume (Hoseney et al 1969). The individual additions of 0.6% and 0.8% free polar lipids and 0.8% bound lipids gave loaf volumes similar to the unextracted control flour (Hoseney et al 1969).

2.33 Free Flour Lipids in Baking

Free lipids constitute 0.8% of wheat flour lipids and are composed of 0.6% nonpolar lipids and 0.2% polar lipids (Hoseney et al 1970). Wheat flour lipids are also composed of 0.6% bound lipids (Hoseney et al 1970). Daftary et al (1968) found that free polar lipids provided more loaf volume improvement than did bound polar lipids, nonpolar lipids, and total free lipids when incorporated into original and defatted flours. They also found that as the polar (particularly free polar lipids) to nonpolar lipid ratio decreased so did loaf volume. Loaf volume improvement for adding the following fractions to unextracted flours in descending order is; free polar lipids, free total lipids, bound polar lipids and lastly free nonpolar lipids (similar to the control) (Daftary et al 1968). Bound polar lipids and total free lipids improved loaf volume to a smaller degree than did free polar lipids (Daftary et al 1968). The nonpolar free lipid fraction reduced loaf volume when incorporated into defatted flours but did not affect original unextracted flours (Daftary et al 1968). The effect on loaf volume of adding the following fractions to petroleum extracted flours in descending order is; free polar lipids, total polar lipids, total free lipids, and lastly free nonpolar lipids (Daftary et al 1968). Shortening additions at 3% (fwb) improved loaf volume response for all lipid classes in petroleum-ether extracted flours (Daftary et al 1968).

2.34 Effect of Flour Lipid Extraction Solvents in Baking

MacRitchie and Gras (1973) found that water saturated n-butanol, acetone/water (90/10), ethanol/water (90/10), acetonitrile, ethanol/water (95/5), or ethanol/ether/water (40/40/20) treatment of flours increased time required to achieve peak development. Thus, these solvents also affect the gluten fraction during lipid extraction. The same study found that chloroform, benzene, petroleum ether, and ethyl acetate did not have any effect on mixing time (MacRitchie and Gras 1973). Reconstitution of flours extracted with petroleum ether resulted in no loaf volume or crumb grain changes while water saturated butanol resulted in severely reduced loaf volumes (Pomeranz et al 1968).

2.4 Wheat and Flour Aging Studies

2.41 Post-Harvest Changes

Freshly harvested wheat goes through an aging process between harvest and milling (Posner and Deyoe 1986). It is characterized by changes in moisture content due to free water forming on the grain as it is stored before milling (Posner and Deyoe 1986). This process, often called "sweating" is thought to affect the baking quality of flour from new crop wheat (Posner and Deyoe 1986). Baking and milling quality have been found to improve as wheat ages (Wang and Flores 1999; Shelke et al 1992a). Wheat storage at lower temperatures and low moisture contents retards the changes brought on by sweating (Posner and Deyoe 1986). Consequently wheat stored at low moisture contents becomes ready for milling at a slower rate than does high moisture wheat (Wang and Flores 1999).

Storing wheat overtime has long been known to improve baking quality (Shellenberger 1939). Shellenberger (1939) found that as new crop hard wheat aged, the loaf volumes did not change significantly during 6 months of storage. Baking quality appeared to be best at 3.5 months of wheat storage, a period similar to what Posner and Deyoe (1986) determined (Shellenberger 1939). Interestingly, maximum germination percentage and dough absorption also occurred at 3.5 months of storage (Shellenberger 1939).

Although baking strength and quality improve as wheat ages, deterioration eventually starts to occur (Fisher et al 1937). Early studies (Fisher et al 1937) found that after deterioration a second improving phase occurs followed by degradation in quality. The rate and severity of

these wheat and flour changes are dependent on the type of wheat, flour grade, bran content, moisture content, storage temperature, relative humidity and microbial activity (Fisher et al 1937). High grade (strong) flours had improved baking quality even after 2 years of storage while weaker flours deteriorated severely (Fisher et al 1937). The same work found that at an average moisture content of 13.2%, flour aged over an 18 month period resulted in minimal improvements in dough and loaf quality.

Flour from new crop hard wheat has been shown to produce low volume loaves (Wang and Flores 1999). Their doughs have poor gas retention, low mixing tolerance, and low water binding capacity (Wang and Flores, 1999). Also, poor texture and crumb grain are common when using freshly milled flour (Pyler 1988). Improved grain crumb and crust characteristics were observed by aging both flour and wheat (Posner and Deyoe 1986).

Untreated freshly milled flour begins the "sweating" process 5 days after milling. This lasts approximately 3 weeks (Pyler 1988). When flour from new crop hard winter wheat stored at 21˚C was baked immediately after milling and again 3 weeks later, no significant changes in loaf volume, water absorption or mixing time with flour or wheat age were seen (Posner and Deyoe 1986). However, flour from new crop HRW wheat from the subsequent crop year (1984) had an initial loaf volume of 836 cc after 3 weeks of aging but a peak volume of 925 cc 126 days later (Posner and Deyoe 1986). Improvements in subjective values (e.g. smooth crust, uniform crumb grain) were also noted with increased wheat age (Posner and Deyoe 1986). However, freshly milled hard wheat flour stored at 20˚C showed an improvement of 88ml in loaf volume after 10 days of storage (Chen and Schofield 1996). Loaf volume stayed constant over an additional 30 days of room temperature (20˚) storage (Chen and Schofield 1996). Yoneyama et al (1970) stored freshly milled refined HRS flour at -30˚C, 0˚C, and 30˚C. They found that specific volume decreased over 60 days of flour storage at 30˚C while specific volume remained constant at 0˚C and -30˚C (Yoneyama et al 1970). The maturation process occurred at a faster rate at 30˚C than it did under storage at -30˚C and 0˚C (Yoneyama et al 1970). Dough elasticity and viscosity increased over time at an accelerated rate at 30˚C compared to changes at the lower storage temperatures (Yoneyama et al 1970).

Daftary and Pomeranz (1965) stored hard red winter and soft red winter wheat for 6 months at 4˚C and aged the wheat for 160 days at 49˚C and 22% moisture. Total polar lipids were extracted with water saturated 1-butanol which were then separated into polar and nonpolar

fractions (Daftary and Pomeranz 1965). The polar lipids resulted in a larger decrease (0.40%, HRW and 0.52%, SRW) after 160 days than did the neutral lipids (0.32%, HRW and 0.34%, SRW) (Daftary and Pomeranz 1965). The HRW and SRW lost 63.49% and 67.53% of their polar lipids respectively after 160 days of storage (Daftary and Pomeranz 1965). The HRW and SRW lost 25.40% and 23.13% of their neutral lipids respectively (Daftary and Pomeranz 1965). The polar lipid content was greater for wheat stored at 4˚C after 140 days (0.67%, HRW and 0.77%, SRW) than storage at 49˚C (0.38%, HRW and 0.39%, SRW) (Daftary and Pomeranz 1965). Digalactosyl glyceride and monogalactosyl glyceride lipids in SRW decreased by 0.10% and 0.05% respectively after 132 days of storage (Daftary and Pomeranz 1965). The nonpolar lipids extracted from SRW wheat showed a decrease in triacylglycerides (0.46%) and an increase in free fatty acids (0.19%) over the same time (Daftary and Pomeranz 1965).

Zia-Ur-Rehman and Shah (1999) stored freshly harvested hard wheat at 10˚C, 25˚C, and 45˚C for 6 months and observed changes in the wheat. They found that storage temperatures of 25˚C and 45˚C resulted in decreased alpha amylase activity, wheat moisture, water soluble amylose, pH, lysine and protein digestibility and an increase in insoluble amylose and titratable acidity while wheat stored at 10˚C resulted in no significant changes to any of the previously mentioned parameters. Wilkes and Copeland (2008) stored wheat at 4˚C and 30˚C for 270 days and found that wheat stored at 30˚C had higher amounts of soluble high molecular weight glutenins.

Skarsaune et al (1970) found that as wheat ripens more ester bonds are formed between glycerol and FFA. This increases the amount of triacylglycerols present in the kernel and leads to a decrease in FFA and monoaclyglycerol content. Linoleic acid increased with kernel ripening while the glycolipid fraction showed no consistent pattern of increase or decrease in concentration (Skarsaune et al 1970).

The new crop wheat phenomenon exists in soft wheat as well. Shelke et al (1992a) milled soft wheat of increasing ages and then aged the flour to determine cake baking quality as a function of both. Shelke et al (1992a) found that cake baking quality parameters such as distilled water binding capacity (DWBC) (Shelke et al 1992b), cake volume and symmetry increased with postmilling flour age of chlorinated flour while gummy cake bottoms decreased. As wheat aged, quality parameters such as high DWBC values, even and positive cake symmetries, high cake volumes and the elimination of gummy cake bottoms developed at an

accelerated rate with postmilling flour age (Shelke et al 1992a). Wheat age did not affect how batter specific gravity changed with flour age but chlorinated flour became stable (no further change) faster than did untreated flour (Shelke et al 1992a). Thus, cake baking quality of freshly milled flour from aged wheat and of new crop wheat was initially low but improved over both flour and wheat age thus verifying that a new crop wheat phenomenon exists for soft wheat (Shelke et al 1992a).

2.42 Flour Storage Studies

Time related changes in flour free fatty acid (FFA) concentration do not appear to affect flour baking quality significantly (Clayton and Morrison 1972). Correlations between increased FFA content and decreased baking quality did exist over flour storage time but many other factors could be affecting the results (Clayton and Morrison 1972). Flour showed the greatest increase in FFA concentration initially after milling. This period also corresponded to a period of improved baking quality (Warwick et al 1979). That said, factors including storage conditions, moisture contents, atmospheric composition (Brandoline et al 2012), other lipid classes, and non-lipid components may lead to the degradation of stored flour after milling (Clayton and Morrison 1972; Warwick et al 1979).

Clements and Donelson (1982) found that throughout cake flour storage the free lipid fraction was altered which correlated with higher cake volumes. Unbleached cake flour (Logan, 50% patent) exposed to moving air for 9 weeks during storage produced cake volumes similar to bleached cake flour. Cake volume increased with flour age after incremental testing (0, 2, 4, 6, 8, 9 weeks) using the previously mentioned unbleached flour. Unbleached cake flour (Arthur, 50% patent) exposed to moving air for 14 weeks was defatted with hexane and baked without lipids and with reconstituted free lipids from 0 week old flour, bleached flour, and 14 week old flour. The flour was baked to determine oven expansion and final cake height (Clements and Donelson 1982). The final cake heights were approximately 2.0 (no lipid), 2.2 (0 week), 4.5 (bleached), and 4.0 (14 week) centimeters, respectively, with the 14 week lipid imparting a higher oven expansion than the bleached lipid (Clements and Donelson 1982). The conclusion was that changes in the free lipid fraction played a role in the increased cake quality of aged flour (Clements and Donelson 1982).

Clayton and Morrison (1972) stored high and low grade flours at -20˚C, aged the flour at 15˚C for 6 months and then carried out lipid analyses. They also studied the lipid content of high grade flour stored for 4 months at 25˚C and low grade flour stored at 37˚C for 3 months. Low grade flours, with higher amounts of bran and germ than the high grade flours contained more glycolipids and neutral lipids (Clayton and Morrison 1972). Flour storage resulted in hydrolysis of MGDG to MGMG and DGDG to DGMG in the low grade and high grade flours at all storage conditions, due to the lipases present in the flour (Clayton and Morrison 1972). The hydrolysis of DGDG and MGDG during storage almost doubled the amounts of DGMG and MGMG (Clayton and Morrison 1972). The total extractable lipid contents and lipid composition stayed constant for all flours due to minimal total degradation of the lipids into water soluble components for all storage conditions (Clayton and Morrison 1972). Warwick et al (1979) also noticed a loss of DGDG and MGDG and a corresponding increase in DGMG and MGMG upon analysis after 0, 18, 36, and 54 months of storage of medium grist flours at 12˚C. The author suggested that these losses were minimal and would not be expected to cause a decrease in baking quality but presents no basis for that conclusion (Warwick et al 1979). Warwick et al (1979) found a loss in total nonstarch fatty acids after 54 months of storage at 12˚C showing that prolonged flour storage is needed to see this change. The amount of free fatty acids present in the flour increased with storage while triacylglycerol concentration fell (Clayton and Morrison 1972; Warwick et al 1979). Free fatty acids initially increased rapidly but the change leveled off after 18 months of storage (Warwick et al 1979). Over time, weak flour had the highest concentration of free fatty acids (Warwick et al 1979). Lipoxygenase and other fatty acid lipid degradation products were not found to be present in this study (Clayton and Morrison 1972). Warwick and Shearer (1980) aged weak, medium, and strong white flours for 5 years at approximately 12˚C. They found that lipoxygenase was responsible for the breakdown of polyunsaturated fatty acids (linoleic acid) during flour storage which explained the loss of total fatty acids after extended storage found by Warwick et al (1979) study. Lipoxygenase converted free fatty acids into lipid hydroperoxides which were enzymatically broken down into monohydroxy-octadecadienoic acid methyl ester (MHA) and dihydroxy-octadecenoic acid methyl ester (DHA) (Warwick and Shearer 1980). The concentration of these hydroxy acids increased linearly throughout flour storage and corresponded to an increase in free fatty acid production in the Warwick et al (1979) study. The concentrations of MHA and DHA in the weak and medium flours were

approximately twice that of the strong flour throughout 60 months of storage (Warwick and Shearer 1980).

Maraschin et al (2008) stored freshly milled straight grade flour at 22˚C, 32˚C, and 45˚C at 3 different moisture contents (10, 12, and 14%). As the storage temperature, moisture, and storage time increased, the amount of lipids hydrolyzed increased, resulting in more free fatty acids and a decrease in triacylglycerols (Maraschin et al 2008). Higher moisture contents and storage temperatures reduced wheat lipoxygenase activity after 16 weeks of storage (Maraschin et al 2008). Flour at 14% moisture stored for 16 weeks at 22˚C, 32˚C, and 45˚C reduced lipoxygenase activity by 34%, 75%, and 90-100%, respectively (Maraschin et al 2008).

Lipoxygenases oxidize polyunsaturated fatty acids (PUFA) most rapidly in the free fatty acid or the monoacylglycerol form (Maraschin et al 2008). Linoleic acid is the main PUFA present in triacylglycerols and therefore the main target of wheat lipoxygenase (Tait and Galliard 1988; Hidalgo and Brandolini 2012). As wheat lipoxygenase becomes more active, fewer μmol/g of oxidative PUFA are available (Maraschin et al 2008). As more oxidable PUFA is present, the rate of oxygen uptake increases. Wheat lipoxygenase uses molecular oxygen to oxidize the PUFA (predominately linoleic or linolenic fatty acids) (Maraschin et al 2008). As wheat lipoxygenase becomes more active, oxygen uptake decreases due to the limited amounts of PUFA present in the flour (Maraschin et al 2008). As wheat lipase activity increases due to higher wheat moisture and storage temperature, wheat lipoxygenase activity decreases (Maraschin et al 2008). Flour at lower moisture contents may have higher lipoxygenase activity but there will be fewer PUFA available for reaction due to limited lipase activity (Maraschin et al 2008). The same study found that bread loaf volume decreased with increasing storage temperature (Maraschin et al 2008). The flour moisture did not result in significant differences in loaf volume until storage was at 45˚C. The initial loaf volume was compared to the loaf volume of flour aged for 16 weeks (Maraschin et al 2008).

Bell et al (1979) found that weak, medium and strong flours (10.3, 12.3, and 13.6% protein respectively) baked every 3 months between 3 and 66 months of storage at -20˚C under N_2-H_2 (95:5) produced bread volumes similar to bread made with freshly milled flour. Weak, strong, and medium flours stored at 12˚C baked every 3 months with 0.7% shortening added produced larger bread volumes than did bread baked without shortening every 6 months over 66 months (Bell et al 1979). The strong flour produced the largest loaf volumes followed by

medium and weak flours, respectively (Bell et al 1979). Medium flour stored at 25˚C baked monthly over 24 months displayed the sharpest decrease in bread volume than did medium flour stored at 12˚C (Bell et al 1979). The same flour also showed the sharpest increase in free fatty acid formation during monthly testing over 2 years (Bell et al 1979). Weak flour stored at 12˚C produced the most free fatty acids over 66 months followed by the medium and strong flours, respectively (Bell et al 1979). Free fatty acid formation did not provide a perfect correlation to bread volume stability over flour storage (Bell et al 1979). Weak flour generated the most free fatty acids but the loaf volumes were more stable during flour storage (Bell et al 1979).

Loaf volumes of freshly milled flour stored at higher temperatures would be expected to increase with time. The maturation process, which is expected to lead to higher loaf volumes, would be accelerated. That said, bread loaf volume reduction appears to correlate with an increase in FFA content. DGDG and MGDG losses and corresponding increases in DGMG and MGMG upon flour ageing at 12˚C (Warwick et al 1979) could be accelerated with increased storage temperature and moisture content. Negative effects in baking quality were noted.

In all, Posner and Deyoe (1986) and Chen and Schofield (1996) found that freshly milled hard wheat flour showed an improvement in loaf volume during storage. Shelke et al (1992a) found that cake baking quality improved with post-harvest flour age. While Yoneyama et al (1970) found that although flour maturation occurred faster at higher storage temperatures, specific volume decreased over 60 days of flour storage at 30˚C.

2.43 Whole Wheat Flour Storage

Whole wheat flour baking quality is known to decrease over time at a faster rate than that of white flour (Tait and Galliard 1988). The lipase in the bran creates free polyunsaturated fatty acids (PUFA) from triglycerides present in the whole wheat flour (Tait and Galliard 1988; Galliard 1986a). Upon hydration lipoxygenase, present primarily in the germ, oxidizes PUFA and to a lesser degree fatty acids present in tri, di, and monoacylglycerols (Galliard 1986a; Tait and Galliard 1988). Galliard (1986b) determined that fatty acid content and O_2 uptake increased over time at +20˚C and that after hydrating a 70:30 bran-germ blend, lipoxygenase oxidized the free PUFA and formed polar hydroperoxy and hydroxy acids. Whole wheat flour stored at +20˚C for 20 weeks was mixed into doughs and aqueous suspensions of 4.8 ml 100 mM Na phosphate buffer (pH 6) and [1-^{14}C] linoleic acid (0.5 μCi: 2.39μg) in 5μl ethanol to determine

the reaction products between lipoxygenase and PUFA (Tait and Galliard 1988). Tait and Galliard (1988) found that whole wheat flour aged 7 months contained 5 times more conjugated oxidized products than did freshly milled flour (when mixed as an aqueous suspension). Thus, stored whole wheat flour had more FFA present for reaction with lipoxygenase and, in consequence, higher amounts of monohydroperoxyoctadecadienoic acid (MHPA) and low amounts of monohydroxyoctadecadienoic acid (MHA) and trihydroxyoctadecenoic (THA) were present (Tait and Galliard 1988). Conversely, freshly milled whole wheat flour had more MHA and THA present and lower amounts of MHPA (Tait and Galliard 1988). The buildup of MHPA from linoleic acid in stored flour is thought to slow it's conversion to MHA (Tait and Galliard 1988).

The flour's lipid fraction is known to play a role in changing the breadmaking quality of whole wheat flour (Barnes and Lowy 1986). Whole wheat milling increases the amount of neutral lipids compared to polar lipids in flour due to the germ and aleurone layers containing higher percentages of neutral lipids (Larsen et al 1989). Lower loaf volumes were found with flour containing higher amounts of free and neutral lipids (Larsen et al 1989).

Wholemeal flour contains, in addition to the starchy endosperm, the bran and germ components. These interact during storage to result in deterioration (Barnes and Lowy 1986; Galliard 1986). When freshly milled whole wheat flour was stored for 20 weeks at +20˚C and -20˚C (Barnes and Lowy 1986), lower bread volumes and higher texture values were observed for wholemeal flour stored at +20˚C than at -20˚C (Barnes and Lowy 1986; Galliard and Collins 1988). Consistent with that, flour stored in cool temperatures had higher loaf volumes than did flour stored at warm temperatures (Wang and Flores 1999). Loaf volumes of whole wheat flour stored at +20˚C and -20˚C followed the same trends for 7 weeks of storage (Barnes and Lowy 1986). Loaf volume for the +20˚C stored flour decreased at a rate of -16.54 ml per week while the -20˚C whole wheat flour volumes decreased at 4.57 ml per week (Barnes and Lowy 1986). The control (-20˚C) white flour had a much higher loaf volume than did the whole wheat flour but its volume still decreased at a rate of -6.05 ml per week (Barnes and Lowy 1986). In most instances, hydrolysis of triacylglycerol to fatty acids contributed to poor baking quality of wholemeal flour (Tait and Galliard 1988). Barnes and Lowy (1986) found that the flour interacted with the germ and bran components during storage at +20˚C for 27 weeks to give a lower loaf volume than when the 3 components were separated during storage until baking. The

storage interactions between the flour, germ, and bran had a greater effect in decreasing loaf volume than did their interactions only during baking (Barnes and Lowy 1986). As the room temperature whole wheat flour aged, the amount of fatty acids increased over 20 week storage (Barnes and Lowy 1986). The increase was pronounced initially but then leveled off (Barnes and Lowy 1986). Conversely the flour stored in the freezer had minimal change in fatty acid content (Barnes and Lowy 1986).

Change in the lipid composition appears to play a role in reducing the baking quality of wholemeal flour (Galliard 1986b). The synergistic effect of the bran and germ when stored together is due to the release of lipases which hydrolyze the triacylglycerols into free fatty acids (Galliard 1986a; Galliard 1986b). The germ releases lipoxygenase which reacts with those fatty acids to start the process of oxidative degradation (Chung et al 1978; Galliard 1986a; Galliard 1986b). Heat treatments can be used to deactivate both enzymes (Galliard 1986b). Fatty acid level and O_2 uptake value increases and decreases were correlated in bran-germ mixtures during 4 weeks of storage at 20˚C (Galliard 1986b). The -20˚C storage condition resulted in little change in fatty acid content and oxygen uptake (Galliard 1986b). When the stored bran-germ mix is composed of more than 30% germ, fewer free fatty acids are present due to the lipase content in the bran being reduced (Galliard 1986b).

In summary, a lipase found in bran that hydrolyzes lipids and results in the deterioration of the baking quality and reduction in loaf volume of whole wheat flour products (Galliard and Gallagher 1988). There are lipids found in bran that lead to a decrease in baking quality of wholemeal flour (Galliard and Gallagher 1988). A general decrease in loaf volume occurs as whole wheat flour ages (Galliard and Gallagher 1988). Structural lipid changes have been associated with the decrease in whole wheat bread loaf volume and are thought to be due to a lipase present in the bran (Galliard and Gallagher 1988).

When extra fat and DATEM are added to aged whole wheat flour the baking quality improved. Aged frozen whole wheat flour showed limited improvement and freshly milled flour did not respond at all (Galliard and Collins 1988). Galliard and Collins (1988) found that the competition for available oxygen between lipoxygenase and ascorbic acid did not affect baking quality improvement. Wheat lipoxygenase could act as an oxidant since it can perform co-oxidation reactions with thiol groups (Galliard 1986a; Galliard and Collins 1988) which increase the mixing tolerance of dough (Hoseney et al 1980).

Bran acts as a diluent of gluten and competes with it for water during dough development (Lai et al 1989c). If insufficient water is added to a whole wheat flour dough, gluten development may be lacking (Lai et al 1989c). Bran particle size did not affect water absorption but finer bran particles decreased mixing time and increased dough strength (Zhang and Moore 1997). Lai et al (1989a) found that fine grinding the bran resulted in quicker water uptake and better loaf quality. Bran of finer particle size is suspected to facilitate lipase contact with the native oils such as triacylglycerols (Galliard 1986b; Galliard and Gallagher 1988). An earlier study by Galliard (1986b) found that decreasing bran size in bran/germ blends increased O_2 uptake and fatty acid content. Bran of decreasing particle sizes stored for 16 weeks at 20˚C resulted in higher fatty acid contents and oxygen uptake which correlated with lower loaf volumes when stored for 1 month before baking (Galliard and Gallagher 1988). Similarly, Noort et al (2010) also found that the incorporation of finely ground bran (75 μm) resulted in bread with smaller loaf volumes (197 ml) than did course bran (600 μm) which gave loaf volumes of 221 ml. Conversely, Pomeranz et al (1977) reported that coarse bran particles lowered loaf volumes more than did finely ground bran. Pomeranz et al (1977) found that added fiber decreased bread loaf volumes due to poor gas retention. Zhang and Moore (1999) found that both the bran ground to coarse (609μm) and to fine (278μm) particles resulted in lower bread specific volumes (6.36 and 6.30 cc/g respectively) compared to the medium (415 μm) ground bran which produced a specific volume of 6.44 cc/g.

Lai et al (1989c) developed ways to produce whole wheat bread with loaf volumes similar to white flour bread. A no-yeast sponge process and a lipoxygenase source (enzyme active soy flour) were used to combat glutathione and methoxylhydroquinone (MHQ) presence in the shorts fraction of whole wheat flour (Lai et al 1989b; Lai et al 1989c). Shorts contain portions of the aleurone layer and 85% of the wheat kernel germ (Lai et al 1989b). Lai et al (1989b) found that certain ratios of MHQ and glutathione decrease baking quality of bread. Lipoxygenase creates peroxides and free radicals that participate in co-oxidation reactions with glutathione and products of MHQ to provide a loaf volume improving effect (Lai et al 1989b). They found that soaking a portion of the bran (Lai et al 1989a) and shorts improved baking quality by adequately hydrating the bran and allowing the lipoxygenase present in the shorts to become active (Lai et al 1989c). Also it was crucial to add as much water as possible to the dough without it becoming too sticky (Lai et al 1989c). A higher content of a NaCl and sodium

citrate blend was used to offset any changes due to the higher ash content in whole wheat flour, which resulted in lower yeast activity, higher loaf volume, improved grain, and better dough handling (Lai et al 1989c). The surfactant SSL was added at a higher level to increase loaf volume and water absorption (Lai et al 1989a; Lai et al 1989c).

2.5 Whole Wheat Milling

Wheat bran color, varietal differences such as water absorption and gluten strength, bran friability, chemical make-up and enzyme levels, the milling process and flour particle size are all factors to consider when producing whole wheat flour (Doblado-Maldonado et al 2012). When milling wheat into refined wheat flour, the separation of the germ, aleurone, and bran layers from the endosperm is necessary to maintain product quality (Posner and Hibbs 2005). This is no longer important in the production of whole wheat flour since all of the kernel components are present in same proportions as found in the wheat kernel. Higher extraction rates result in the flour containing more bran, germ, and increasing amounts of vitamin B, dietary fiber, amino acids, minerals, protein and fat (Dewettinck et al 2008). However, baking quality decreases with higher extraction rates (Dewettinck et al 2008).

Whole wheat flour can be produced using stone, plate, hammer and roller mills all of which affect starch damage and lipid and protein composition (Prabhasankar and Rao 2001). Stone and plate grinding methods are more severe and produce more heat which is correlated to higher starch damage, the binding of free lipids to starch and protein, and protein agglomeration resulting in a reduction of essential amino acids such as lysine (Prabhasankar and Rao 2001). Hammer mills can also produce excess heat and require large amounts of energy (Posner and Hibbs 2005). Roller mill grinding is less severe (Prabhasankar and Rao 2001). The roller mill is the most common and versatile piece of grinding equipment in a flour mill (Posner and Hibbs 2005). Roll diameter, length, surface hardness, taper, differential, hand, and corrugation profile, direction, spiral, and pitch can all be customized for a particular operation (Posner and Hibbs 2005).

2.6 Lipid Deterioration Enzymes and Reactions

There are 3 different ways lipids can deteriorate in flour (Clayton and Morrison 1972). The first, oxidative degradation, occurs below 8% moisture and the second, microbial

degradation, above 15% moisture (Clayton and Morrison 1972). The third, lipid hydrolysis, is most active at 17% moisture and is 50% active between 10% and 14% moisture (Doblado-Maldonado et al 2012). The unstable lipid component in whole wheat flour can deteriorate through both enzymatic and non-enzymatic pathways and thus lipid deterioration is one of the reasons whole wheat flour baking performance and functionality decrease throughout storage (Doblado-Maldonado et al 2012).

2.61 Oxidative Rancidity

Autoxidation, a non-enzymatic pathway, uses molecular oxygen to oxidize unsaturated fatty acids (Pyler 1988). Unsaturated fatty acid chains lack their full complement of hydrogen atoms and double bonds are present (Pyler 1988). These double bonds are reactive sites (Frankel 1980). An especially reactive site occurs when a methylene group is present between 2 of the double bonds (Pyler 1988). Linoleic and linolenic acids possess such isolated methylene groups (Pyler 1988) and are, therefore quite reactive. Autoxidation involves free radicals and proceeds via initiation, propagation, and termination steps (Frankel 1980). Heat or light starts the process by causing the loss of a hydrogen ion usually near a double bond (Pyler 1988). The loss of a proton results in a free radical (a carbon missing an electron) (Pyler 1988). Molecular oxygen then binds to that site creating a peroxide radical but it too then has an unpaired electron (Pyler 1988). The peroxide free radical then takes a hydrogen ion from another reactive site (Pyler 1988) creating a hydroperoxide (Pyler 1988). Hydroperoxides breakdown into secondary products such as hydroxy acids, aldehydes and ketones (Frankel 1980; Pyler 1988). It is these secondary products of the hydroperoxides that are responsible for rancid flavors and odors (Pyler 1988).

Lipoxygenase is a dioxygenase present in plants, animals, and fungi (Feng et al 2010). In the wheat kernel, lipoxygenases are present in the germ and bran (Doblado-Maldonado et al 2012) where they can enzymatically catalyze oxidation reactions (Chung et al 1978; Pyler 1988). This enzyme is most active between a pH of 4.5-6 (Doblado-Maldonado et al 2012). Lipoxygenase catalyzes reactions on cis,cis-1,4-pentadiene structures (Mauch et al 1997) adding molecular oxygen to the isolated methylene groups between 2 double bonds of polyunsaturated fatty acids (Pyler 1988; Mauch et al 1997). Wheat lipoxygenase isoenzymes, L-1, L-2, L-3, and L-a, preferentially react with free fatty acids and monoacylglycerols containing linoleic and

linolenic acids (Chung et al 1978; Shiiba et al 1991). The primary products of this catalyzed reaction are conjugated fatty acid cis, trans-diene hydroperoxides (Mauch et al 1997). The hydroperoxides break down into secondary products such as hydroxy acids (Frankel 1980), ketones, lactones, furans, and aldehydes which can impart rancid odors (Doblado-Maldonado et al 2012). Both autoxidation and lipoxygenase pathways can produce the same secondary products (Doblado-Maldonado et al 2012). Hydroperoxide decomposition is often initiated by the homolyitic cleavage of the O-O bond which can occur thermally or via metal-mediated catalysts (Kubow 1992). The reduction of hydroperoxides to hydroxy acids may affect dough rheology by oxidizing sulfhydryl groups into disulfide bonds (Shiiba et al 1991; Chung et al 1978). Fatty acid radicals produced by lipoxygenase oxidize carotenoids and tocols, destroy nutritious unsaturated fatty acids, and can denature proteins (Doblado-Maldonado et al 2012). The loss of carotenoids is detrimental during pasta production where a yellow color is desired (Hidalgo and Brandolini 2012) but oxidation also produces whiter bread crumb which is positive result (Hidalgo and Brandolini 2012). Metal ions Cu^{+2}, Hg^{+2}, Mn^{+2}, and Fe^{+2} (Shiiba et al 1991) and high heat and moisture (Maraschin et al 2008) reduce lipoxygenase activity in wheat.

2.62 Hydrolytic Rancidity

Lipases are another common enzyme present in wheat (Pyler 1988). They are produced by bacteria or fungi (Arpigny and Jaeger 1999). Lipases hydrolyze fatty acids that are esterified to glycerol, preferably triacylglycerol (Pyler 1988; Arpigny and Jaeger 1999). They hydrolyze tri, di, and monoacylglycerols to produces various free fatty acids and mono and diacylglycerols (Urquhart et al 1984). These products can then be oxidized to create rancid odors (Pyler 1988) and bitter flavors in whole wheat flour (Doblado-Maldonado et al 2012). Free fatty acids with fewer than 14 carbons are major contributors to rancidity (Pyler 1988). Lipases generally target specific esterified fatty acids of triacylglycerol (Moayedallaie et al 2010). Although Clayton and Morrison (1972) determined, based on the products recovered after lipolysis, that it was possible for them to be generated either randomly or specifically. The DGDG fatty acids appeared to be cleaved without any specificity (Clayton and Morrison 1972).

If lipase activity can be reduced, the amount of substrate (polyunsaturated free fatty acids) available for lipoxygenase would be limited and the shelf life of flour may be extended (Doblado-Maldonado et al 2012). Polyunsaturated free fatty acids can also reduce the amount of

lipid binding to the gluten proteins during mixing which hinders the gas retaining properties of the lipoprotein complex in wheat flour doughs (Doblado-Maldonado et al 2012). Methods to reduce lipase activity have utilized acid, heat, ethanol vapor, and gamma radiation treatment and the addition of metal ions ($FeCl_3$) (Doblado-Maldonado et al 2012). While all of the previously mentioned methods can reduce lipase activity, they all have drawbacks. Heat treatment and the addition of salts may promote autoxidation in flour (Doblado-Maldonado et al 2012). Treatment with ethanol vapor extracted antioxidants from rice bran and acid treatment may affect flour functionality (Doblado-Maldonado et al 2012). Flour treated with gamma radiation may not be accepted by consumers. Interestingly, exogenous lipases can improve loaf volume by hydrolyzing fatty acids from triacylglycerols, DGDG, and phospholipids there by increasing their polarity. A majority of the lipase enzymes added to a standard bread formula increased loaf volume to the same degree as does DATEM (Moayedallaie et al 2010).

2.7 Mass Spectrometry

Electrospray ionization tandem mass spectrometry can be used to quantitatively determine lipid composition in mediums such as flour and plant membranes (Finnie et al 2009; Welti et al 2002). Polar lipids can easily be analyzed because the polar head group is ionized into a common fragment that is either charged or neutral thus making signals easier to interpret (Welti and Wang 2004). Mass spectrometry signals are also used to identify the fatty acids present by correlating chain length and number of double bonds to the fatty acids present in the sample (Welti and Wang 2004). The technique can quickly analyze and process large quantities of lipid samples in a short time (Welti et al 2002). Lipid profiling using mass spectrometry can help determine how lipids function in processes such as gas cell stabilization in baking (Finnie et al 2009), plant defense responses (Welti and Wang 2004), stress response, plant growth, and development (Welti et al 2002), and other cellular biochemical activities such as lipid metabolism (Divaiah et al 2006).

Chapter 3 - Materials and Methods

3.1 Wheat Samples

Hard red winter wheat, variety Overley, was grown at Galva, KS in the 2011 crop year. One sample of 159 kg (350 lbs.) was obtained from this location and used in subsequent studies. As described in Figure 3.1, the freshly harvested wheat was aged at 2 different temperatures RT (23˚C) and FZ (-26˚C) for 8, 50, and 91 days before milling. At the end of each wheat aging period, whole wheat flour (WWFL) was milled from both RT and FZ wheats. Those flours were then aged for 31 days at 74˚F and -15˚F. Bread baking and lipid extraction studies were performed for each wheat milling flour series.

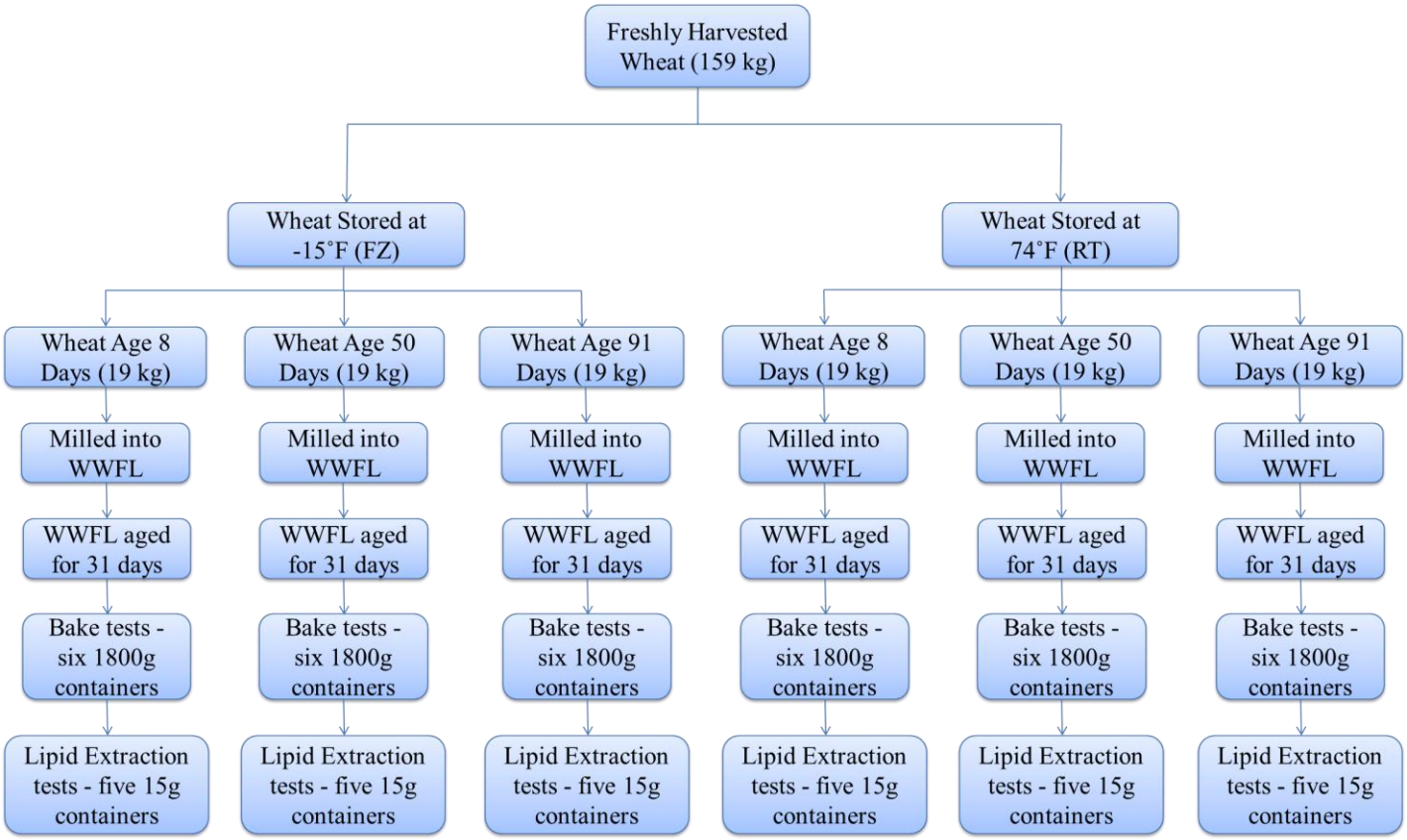
Wheat was collected 3 days after harvest and immediately stored in a lab freezer for 3 days at -15˚F to kill any insects present. The wheat was then divided into the FZ and RT treatment groups. Four containers (19 kg each) of Overley wheat were stored at RT in the Flour and Dough Testing Lab (Waters Annex 103) at a temperature of approximately 74˚F. Four 19 kg containers and 1 7.7 kg container were stored in the Bake Lab (SH 110) freezer at -15˚F. One bag of wheat (to be milled 8 days after harvest) was taken from each treatment group and placed directly in the Milling Lab (SH 101) to equilibrate to RT for 1 day at 74˚F. This left 3 bags of wheat in Waters Annex 103 and SH 110. The FZ and RT 8 day wheat samples had been subject to the same temperature conditions (at the farm site for 3 days at ambient temperature, in the freezer for 3 days, and at RT for 2 days) up to the first milling so both treatment groups were identical. Dividing into FZ and RT conditions and the equilibration of the 8 day wheat to RT occurred on the same day. After the wheat equilibrated to RT, it was cleaned and its moisture determined as described in section 3.2. Wheat samples were then milled into WWFL and blended on the 9th day after harvest as per Figure 3.4 and described in section 3.3. The WWFL from the FZ wheat was stored in the SH 110 freezer at -15˚F. The WWFL from the RT wheat was stored in the Waters Annex 103 at 74˚F. WWFL moisture content for all treatment groups was determined as per section 3.2. Next, the WWFL granulation and flour particle size were determined as per section 3.4. The WWFL was malted as described in section 3.4. WWFL was divided for lipid extraction (LE) and protein analysis. The flour used for lipid extraction and

protein analysis was removed before malt addition to prevent any variation due to the barley lipids and protein.

WWFL was also sub-divided into containers for mixograph® analysis, test mixing, test baking studies, and for the actual baking tests. The mixograph® was used to identify a range of probable water absorptions and mixing times for the WWFL as per section 3.5. Full scale test mixing studies were performed in the presence of all the formula ingredients to narrow the range of possible mixing times and water absorptions per section 3.5. Finally, test baking studies were used to determine the final water absorptions and mixing times per section 3.5. Both FZ and RT WWFL samples were baked 19, 22, 25, 28, and 31 days after milling per section 3.5. The FZ and RT WWFL lipids were extracted 20, 23, 26, and 29 days after milling per section 3.6. Specific details of the experiment are described in the following sections.

By the time the baking and lipid extraction experiments were completed for the 8 day wheat there was a 6 day break remaining before the FZ 50 day wheat was allowed to equilibrate to RT for 2 days. RT and FZ wheat moisture was determined and the process (detailed above) was repeated from this point. The 50 day wheat studies were completed so as to allow a 7 day gap before the FZ 91 day wheat was allowed to equilibrate to RT for 2 days. The RT and FZ wheat moisture was determined and the process repeated.

Figure 3.1 Table Experimentation Flow



3.2 Wheat Physiochemical Tests

Wheat was cleaned using the Carter Dockage Tester (Carter-Day Company, Minneapolis, MN). Wheat moisture was determined as per the American Association of Cereal Chemists International (AACCI) approved method 44-15.02 (AACCI, 2009). The RT and FZ wheat moisture was analyzed in triplicate. The Falling Number AB machine grinding gap was set to zero and wheat was ground to a uniform degree for moisture analysis.

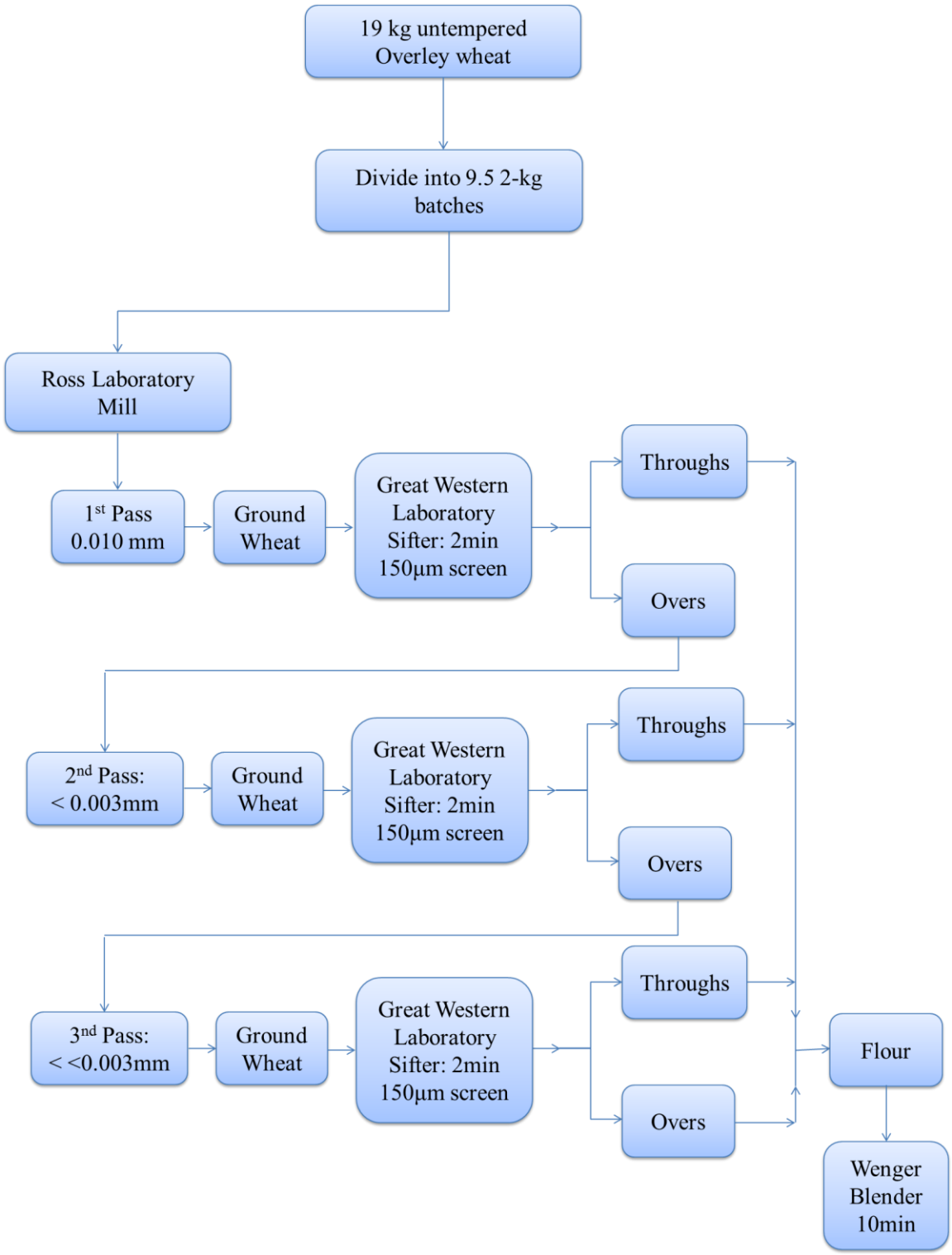
3.3 Wheat Milling

Wheat stored in the freezer was brought to room temperature 2 days prior to milling. The wheat was left untempered in order to make the bran more prone to shattering and thus reduce its particle size. The wheat was milled into whole wheat flour using a single corrugated Ross table top mill stand. A flow sheet (Figure 3.4) and procedure outlined in Stoerzinger (2009) was used. A single blue roll stand, as seen in Figure 3.2, was used with a differential of 2.5:1, 0.5 inch spiral, 24 corr/in pitch, and a grinding action of dull:dull. Approximately 19kg of FZ Overley wheat was milled on the Ross table top mill. The room temperature and relative humidity were recorded. As described in Figure 3.4, 2 kilogram aliquots were weighed and sent through the first pass. The feed rates for all 3 passes were recorded in g/min and the same roll stand was used for all 3 grinding steps. The roll gap for the first pass was set to 0.010mm. The ground wheat was then sifted using a Great Western Sifter (Figure 3.3) for 2 minutes over a 150μm screen. Three triangle pan cleaners were placed on top of the 150μm sieve. The throughs went to flour and the overs to the second pass. That roll gap was set to < 0.003mm. The ground material was again sifted for 2 minutes over the 150μm screen. The throughs went to flour and the overs to the third pass. The roll gap for the third pass was set to <<0.003 so that the rolls were set as tight as they could be without choking them. The ground material from the third pass was either sifted or sent directly to flour. Both the overs and throughs of this step went to flour. The total flour was collected and blended in a Wenger mixer (capacity, 175 lbs.) for 10min. The flour was left unbleached and not enriched. After blending the flour was bagged and returned to the appropriate storage temperature.

Figure 3.2 Table Top Ross Roll Stand

Figure 3.3 Great Western Sifter

Figure 3.4 Whole Wheat Flour Milling Flowsheet



3.4 Flour Physiochemical Tests

Flour moisture content was determined using AACC method 44-15.02 (AACCI, 2009). The RT and FZ flour moisture content was analyzed in triplicate. Falling Number (AB 1400, Stockholm, Sweden) analysis followed AACC method 56-81.03 (AACCI, 2009). Flour was first tested for naturally occurring alpha amylase (both RT and FZ whole wheat flour). Diastatic malted barley flour was then added until a falling number between 250 and 300 seconds was reached. An optimized level between 250 and 270 seconds was achieved for all flour samples. Once an appropriate malt level was found, the flour was tested in triplicate at that level to ensure the reading was between 250 and 300 seconds. Flour was scaled on 14% moisture basis.

Flour protein content was assessed by nitrogen combustion (N% x 5.70) using AACC method 46-30.01 (AACCI, 2009) (Leco Corp. St. Joseph, MI). The RT and FZ flour protein content was analyzed in triplicate. Flour granulation was determined using the Ro-Tap Testing Sieve Shaker Model 1B (Tyler Industrial Products) in Figure 3.7, the Alpine Air Jet Sieve (Alpine, Augsburg, Germany) (Hosokawa Alpine Aktiengesellschaft: Alpine operating instructions), and the Fisher Sub Sieve Sizer (Fischer Scientific, Pittsburg, U.S.A.) in Figure 3.6.

Granulation curves for the FZ and RT flours were determined using a modified AACC 66-20.01 Ro-Tap Sieve Shaker method (AACCI, 2009) developed by Stoerzinger (2009). The RT and FZ flour granulation was analyzed in triplicate. The data was displayed as the cumulative percent held over versus aperture opening in microns. One hundred grams of flour (14% MB) was weighed and sifted for 6 minutes with 2 pan cleaners per sieve. The data was recorded as the overs of the #20, 30, 40, 100, and pan. The respective micron openings for the sieves were 841, 594, 420, 250, 150 and < 150. U.S. Standard Sieves from Fischer Scientific Company were used in all cases. The Alpine Air Jet Sieve was also used to determine flour particle size. The RT and FZ flour samples were analyzed in triplicate. A 25 gram sample of blended whole wheat flour was sifted for 5 minutes on a 75μm screen (200 mesh/in). The results were recorded as the percent passing through a 75μm screen. The Fisher sub sieve sizer required 1.44g of flour that had been previously sifted through a 150 μm screen on the Alpine. The average particle diameter of that flour was determined in triplicate. The Fisher sub sieve sizer is based on the principle that air flows more easily through particles of larger size than it does thru

smaller ones. Pressurized air is sent through the flour sample (Fisher Sub Sieve Sizer: Fischer Scientific Instruction Manual). The ease with which the air flows through the sample is indicated by the water level in the manometer. A chart behind the manometer is used to correlate the water level to the sample's particle size (Fisher Sub Sieve Sizer: Fischer Scientific Instruction Manual).

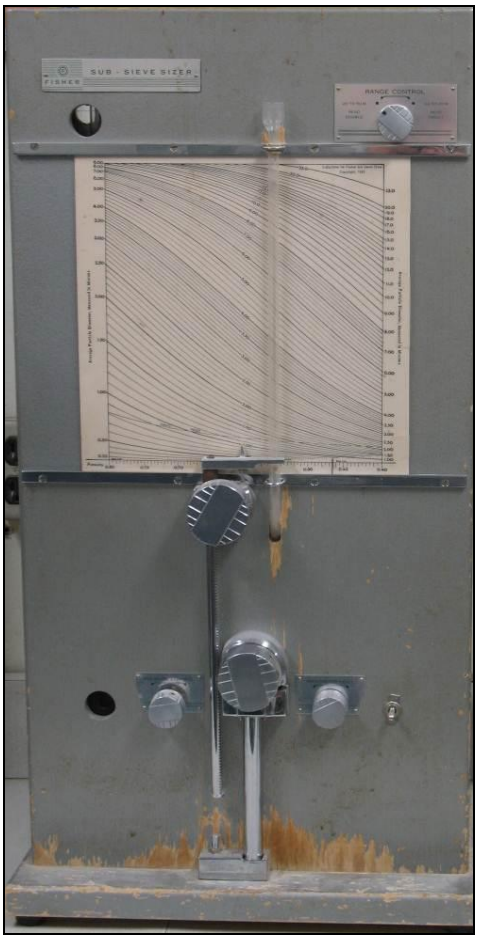


Figure 3.5 Fisher Sub Sieve Sizer

Figure 3.6 Ro-Tap Sieve Shaker

3.5 Baking

The pup loaf test baking procedure was AACC approved method 10-10.03 (AACCI, 2009). This is a straight dough method that uses 90 minute fermentation with the dough sheeted at 3 different intervals (52, 25, and 13 minutes) (National Manufacturing Co, Lincoln, Nebraska). The fermentation cabinet conditions were 84-86˚F at 92-97% R.H. (National Manufacturing Co, Lincoln, Nebraska). The target was to achieve an 84˚ wet bulb reading. The moulder was also manufactured by National Manufacturing Co, Lincoln, Nebraska. Each loaf was proofed to a height of 7.6 cm before baking in the oven. Whole wheat bread was baked at 419˚F (215˚C) for 24 minutes in a reel oven (National Manufacturing Co, Lincoln, Nebraska). Loaves were allowed to cool for 2 hours then weighed and their volumes measured using rapeseed displacement. The weight and volume data were used to calculate specific volume. Loaves were sliced (15 mm width) the following day using a professional food slicer. Loaves were sliced with the break and shred facing away from the blade. The crumb quality, strength line, break and shred were analyzed subjectively. The middle slice was imaged with C-Cell (CCFRA Technology, Ltd., U.K.) using C-Cell software 2.0. The bread was imaged with the break and shred to the right side.

The formula was: whole wheat flour (100g 14% M.B.), all-purpose shortening (3g) (Ventura Foods, Brea, CA), salt (1.5g) (Top-Flo Evaporated Salt, Cargill), sucrose (6g)

(Michigan Sugar Co., Bay City, MI), instant active dry yeast (2g) (SAF Instant Yeast, Milwaukee, WI), diastatic malted barley flour (AB Mauri Bakery Ingredients, Chesterfield, MO), ascorbic acid (50ppm) (Research Products, Salina, KS), and water (optimum).

Before actual experimental baking, mixograph®, test mixing, and test bakes were performed to optimize water absorption and mixing time. The tests before actual experimental baking helped determine optimum water absorption and mixing time to achieve the maximum loaf volume for the FZ and RT whole wheat flours.

A 10g mixograph® (National Manufacturing Co, Lincoln, Nebraska) was used following AACC method 54-40.02 to determine mixing time and water absorption (AACCI, 2009). Absorptions ranging from 84% (extremely wet) to 68% (extremely dry) were tested. The time to peak height for the midline analysis was used to predict optimum mixing time. The time corresponding to the point where the slopes from the hydration and breakdown part of the curves meet was also used to determine optimum mixing time. Lines were drawn in the center of the mixogram curve along the slope of both the increasing and decreasing segments. The time on the x-axis where the 2 lines met was the optimum mixing time and was usually around the peak of the curve. The results were analyzed and the range of possible water absorptions was narrowed. Water absorptions considered too low had mixograms with steep breakdowns and did not form viscoelastic doughs. Water absorptions considered too high were thin and showed sway back in the hydration part of the curve. (Water absorptions considered too high were 81% and above and anything below 70% was too dry).

Test mixing was performed on treatment groups using 100g pin mixers (National Manufacturing Co, Lincoln, Nebraska). The test mixing studies were performed using the same mixers and formula as the experimental baking tests. The range of water absorptions to be tested was determined by analysis of mixogram curves. Because the range of water absorptions was so wide they were divided into smaller groups where each group was assigned a mixing time which was determined by mixograph® midline analysis (see above). Several mixing series were performed until the ranges of possible optimum water absorptions and mixing times were narrowed. Water absorptions were increased by 1% increments at a constant mixing time until the dough was too sticky to handle. Three to 5 water absorptions below the point where the dough was too sticky were chosen for test baking. Optimum water absorption was achieved when the maximum amount of water was added to flour that formed a dough that could be

handled adequately. Once a range of water absorptions were selected the mixing time was varied for each absorption level. Mixing times that resulted in under and overdeveloped doughs were tested. Doughs that were mixed to optimum went through several changes. First the dough appeared wet, sticky, and stringy and very extensible. With further mixing the dough became drier, more cohesive, and viscoelastic. The dough picked up any remaining dough strands on the side of the bowl and mixing pins. When the dough was overmixed, it became wet and extensible. Mixing times too far below and above the optimum produced low loaf volumes. Therefore, 3 to 4 mixing times were chosen between the point when the dough became a drier viscoelastic mass and overmixed were chosen for test baking. The whole wheat flour dough should be able to be rounded, sheeted, moulded, and panned without excessive tearing and sticking. Ultimately, the only way to know if a dough can be handled is through test baking.

The frozen flour was removed from the freezer the night before baking to allow it to temper to room temperature prior to scaling and baking. Three preliminary test bakes were performed for each treatment group. One test bake was performed at a constant mix time and the second at constant water absorption. The water absorptions and mix times used in the test bakes were determined through mixograph® and test mixing results. The first 2 preliminary test bakes had 16 replicates for each treatment group. A third preliminary test bake was done as needed to further refine the mixing time and water absorption levels. Ten replicates were baked for each treatment group in the third preliminary test bake. The third bake was not carried out on the 91 day wheat as the water absorption and mixing times for the FZ and RT flours were satisfactorily determined after the first 2 test bakes. Based on the preliminary bakes, mixing times and water absorptions to be used in the final experimental bakes were determined. Doughs that were too sticky and slack and tore in the sheeting and moulding steps were not chosen. Loaf volume and crumb texture were also factors. The mixing times and water absorptions that produced bread with the largest loaf volume and had an even crumb without holes were selected for the actual bake tests. Sixteen replicates were baked from each treatment. The final mixing times and % water absorptions chosen for the RT and FZ WWFL bakes are shown in Table F.1.

3.6 Flour Lipid Extraction

The lipid extraction procedure was a modified version of that of Finnie et al (2009). Modifications to Finnie et al (2009) were; the collection of 3 replicates for each lipid class (neutral, glycolipid, and phospholipid) instead of 5 replicates, and the use of 2.4g of whole wheat flour instead of 0.5g. The procedure was carried out at room temperature (approximately 22˚C). Neutral lipids, phospholipids, and glycolipids were extracted from both RT and FZ flour between the bakes that occurred every 3 days.

The procedure itself was performed over 2 days. (Appendix A: Solution Identification) The first half of the procedure involved separating free and bound lipids from whole wheat flour as per Figure 3.7. A sample of whole wheat flour (2.4g) was weighed out and mixed with 24 ml hexane (Flour:hexane ratio of 1:10). The mixture was vortexed until suspended and shaken using the vortex for 1 hour at room temperature after which it was centrifuged at 3000xg for 5 minutes. Centrifuge acceleration and deceleration were set at 9 and 1 respectively for all runs. The supernatant (containing the free lipids) was decanted and discarded. The remaining flour was air dried. The bound polar lipids were extracted by adding 2g of air dried flour in 12 ml solution 6 (isopropanol and water, 90:10). A flour sample to isopropanol and water (90:10) ratio of (1:6) was used. The mixture was then vortexed until suspended and shaken for 1 hour at room temperature before being centrifuged for 5 minutes at 3000xg. The supernatant, containing the bound lipids was decanted and saved. The supernatant was dried and capped under nitrogen and stored at -20˚F. The pellet was discarded.

The second part of the procedure, performed the following day, entailed separating the neutral lipids (NL), glycolipids (GL), and phospholipids (PL) from the previously isolated bound lipids as per Figure 3.8. HyperSep* silica solid phase extraction (SPE) columns with a 5000mg bed weight and 25ml tube volume (Thermo Fischer Scientific) were used. Solid phase extraction columns use less solvent, glassware and sample handling time and have better analyte recovery than open column chromatography (Ohm and Chung 1999). Approximately 3-5 bed volumes of solvent were used for conditioning purposes (Agilent Technologies, Inc. 2011). The mass of the material to be separated in the column was not more than 5% of the sorbent weight (1998 Sigma-Aldrich Co).

The dried bound lipids were dissolved in 4ml of solution 1 (hexane, ethyl acetate, acetic acid, 95:5:0.2) and the resulting solution stirred to disperse any residual lipid or flour material. It was then centrifuged for 5 minutes at 3000xg. The supernatant, containing the dissolved bound lipid was then transferred to a clean test tube. The SPE cartridges were conditioned using 7.5ml of solution 7 (hexane) and 7.5ml solution 1 (hexane, ethyl acetate, acetic acid, 95:5:0.2). The dissolved bound lipids were added when the conditioning solvent was level with the silica bed and the NL eluted with (10ml) solution 1 (hexane, ethyl acetate, acetic acid, 95:5:0.2). The NL collection tube was quickly flushed with nitrogen and capped. The cartridge was then washed with 10ml solution 2 (hexane and ethyl acetate, 95:5), which was discarded. When the wash solvent was level with the silica bed, nitrogen was blown into the column for several seconds to partially dry it. (Note: The column should never run dry during this procedure.) The GL were next eluted using 10ml solution 4 (tetrahydrofuran, acetonitrile, isopropanol, 35:35:30) and the GL collection tube was quickly flushed with nitrogen and capped. The column was again washed with 10ml solution 2 (hexane and ethyl acetate, 95:5) and partially dried. Finally the PL were eluted using (10ml) solution 5 (acetonitrile and methanol, 35:65). The PL collection tube was quickly flushed with nitrogen and capped. NL, GL, and PL fractions were dried at 50˚C under N_2 gas. The samples were then re-dissolved in (500µl) solution 3 (chloroform and methanol, 2:1) to a final concentration of 5mg flour equivalents per microliter (Greenblatt et al 1995).

The dissolved lipid was then centrifuged at 3000xg for 5min and the supernatant transferred to a 4ml HPLC vial. Those samples were flushed with nitrogen, capped and stored at -20˚F.

Two ml HPLC vials were used when preparing samples for the Kansas Lipidomics Research Center Analytical Laboratory for analysis as per Figure 3.9. To calculate dry lipid weight, the tare weight of a 2ml vial was recorded, the lipid samples transferred from the 4ml to the 2ml vials, dried under nitrogen at room temperature and then the vials were weighed and the dry lipid weight was calculated. The lipid samples were then dissolved in 1ml of HPLC grade chloroform, the vials flushed with nitrogen and stored at -20˚F. Before storing in the freezer, the 2ml vials were wrapped with parafilm to help retain the nitrogen.

The glycolipids analyzed in these studies were; monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), digalactosylmonoacylglycerol (DGMG), and monogalactosylmonoacylglycerol (MGMG).

An automated electrospray ionization-tandem mass spectrometry approach was used, and data acquisition and analysis and acyl group identification were carried out as described previously (Devaiah et al 2006) with modifications. An aliquot of 2 to 10 µl of extract in chloroform was used. Precise amounts of internal standards, obtained and quantified as previously described (Welti et al 2002), were added in the following quantities (with some small variation in amounts in different batches of internal standards): 1.01 nmol 16:0-18:0-MGDG, 0.20 nmol di18:0-MGDG, 0.25 nmol 16:0-18:0-DGDG, and 0.36 nmol di18:0-DGDG. The sample and internal standard mixture was combined with solvents, such that the ratio of chloroform/methanol/300 mM ammonium acetate in water was 300/665/35, and the final volume was 800µl.

Unfractionated lipid extracts were introduced by continuous infusion into the ESI source on a triple quadrupole MS/MS (API 4000, Applied Biosystems, Foster City, CA). Samples were introduced using an autosampler (LC Mini PAL, CTC Analytics AG, Zwingen, Switzerland) fitted with the required injection loop for the acquisition time and presented to the ESI needle at 30 µl/min.

Sequential precursor and neutral loss scans of the extracts produce a series of spectra with each spectrum revealing a set of lipid species containing a common head group fragment. Lipid species were detected with the following scans: MGDG, $[M + NH4]^+$ in positive ion mode with NL179.1; and DGDG, $[M + NH4]^+$ in positive ion mode with NL 341.1. The scan speed was 50 or 100 u per sec. The collision gas pressure was set at 2 (arbitrary units). The collision energies, with nitrogen in the collision cell, were +21 V for MGDG, and +24 V for DGDG. Declustering potentials were +90 V for MGDG and DGDG. Entrance potentials were +10 V for MGDG and DGDG. Exit potentials were +23 V for MGDG and DGDG. The mass analyzers were adjusted to a resolution of 0.7 u full width at half height. For each spectrum, 9 to 150 continuum scans were averaged in multiple channel analyzer (MCA) mode. The source temperature (heated nebulizer) was 100 °C, the interface heater was on, +5.5 kV or -4.5 kV were applied to the electrospray capillary, the curtain gas was set at 20 (arbitrary units), and the 2 ion source gases were set at 45 (arbitrary units).

The background of each spectrum was subtracted, the data were smoothed, and peak areas integrated using a custom script and Applied Biosystems Analyst software. The lipids in each class were quantified in comparison to the 2 internal standards of that class. The first and

typically every 11th set of mass spectra were acquired on the internal standard mixture only. Peaks corresponding to the target lipids in these spectra were identified and molar amounts calculated in comparison to the internal standards on the same lipid class. To correct for chemical or instrumental noise in the samples, the molar amount of each lipid metabolite detected in the "internal standards only" spectra was subtracted from the molar amount of each metabolite calculated in each set of sample spectra. The data from each "internal standards only" set of spectra was used to correct the data from the following 10 samples. Finally, the data were corrected for the fraction of the sample analyzed and normalized to the sample "dry weights" to produce data in the units nmol/mg.

Figure 3.7 Lipid Extraction Procedure Day 1

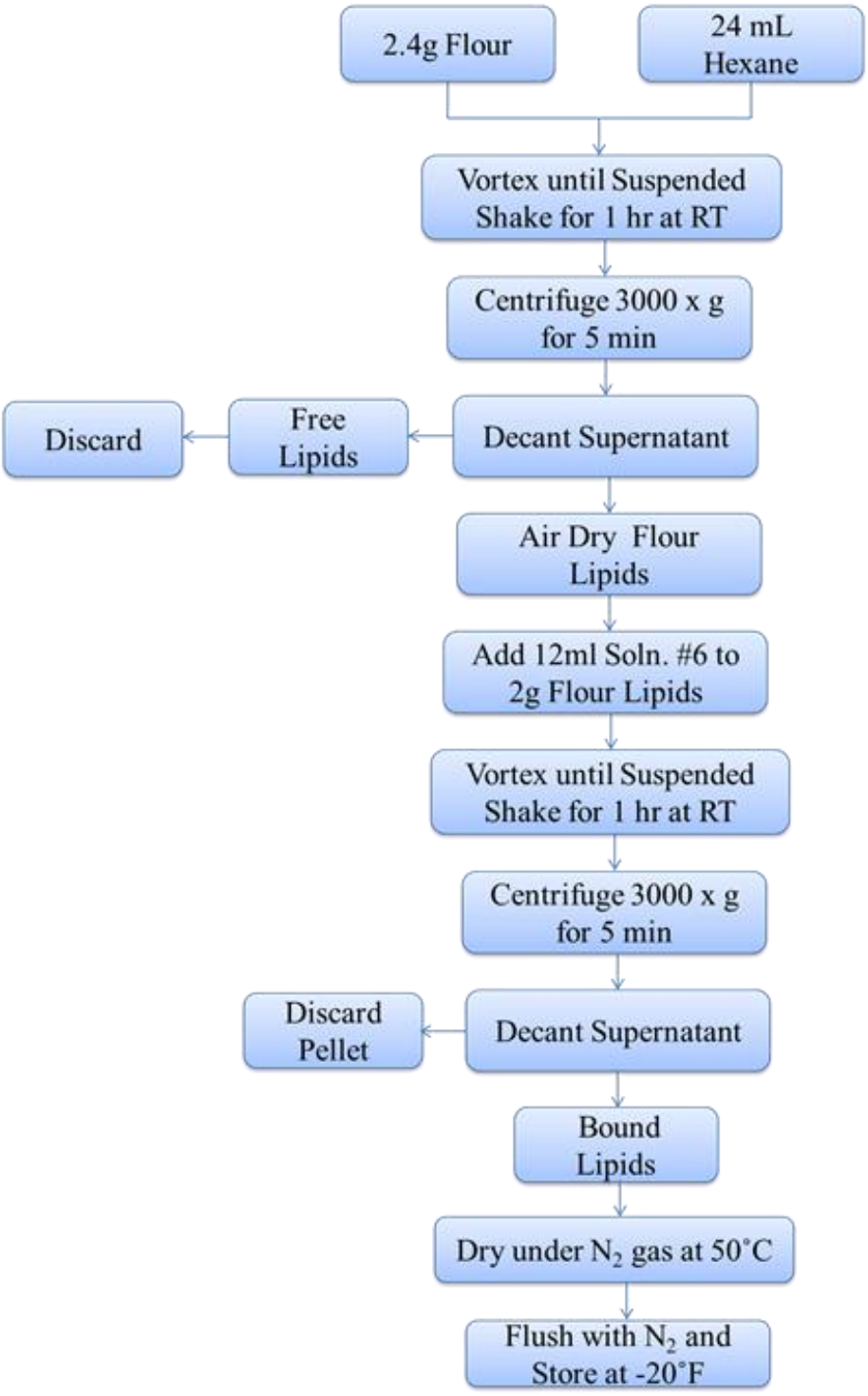
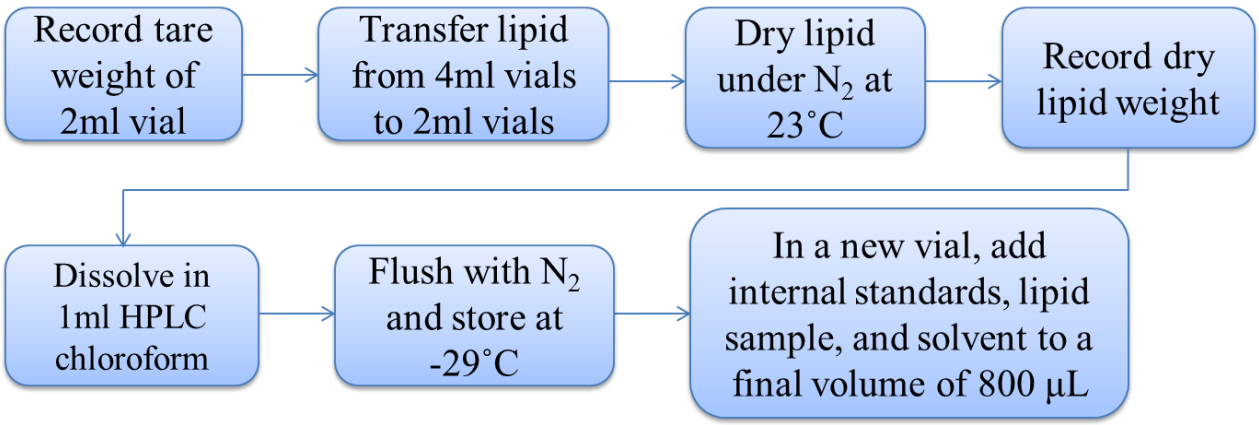


Figure 3.8 Lipid Extraction Procedure Day 2

Figure 3.9 Mass Spectrometer Preparation



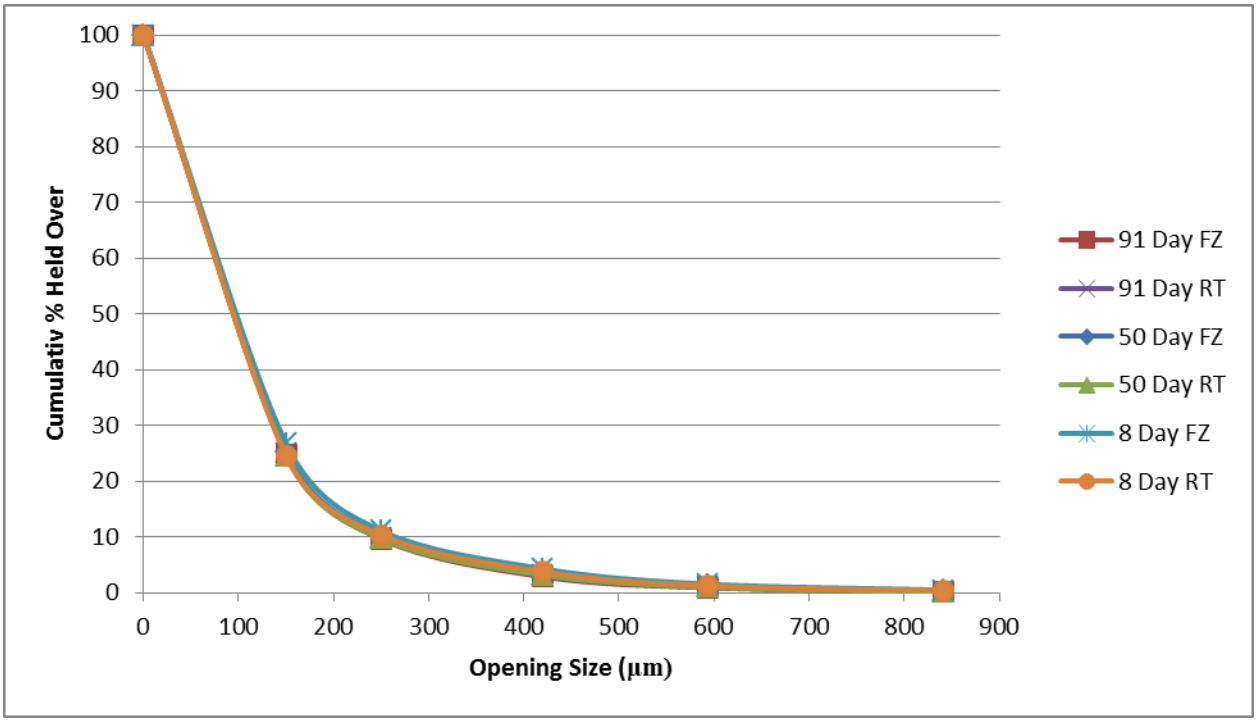
3.7 Statistical Analysis

A completely randomized design was used. Each independent observation (loaves of bread and lipid extract) was compared within each wheat age, flour age and storage condition with each other. It was assumed that the individual loaves of bread and lipid extract were independent observations within each combination of wheat age, flour age and type of storage. Observations at each combination of wheat age, flour age and type of storage were used which resulted in a full factorial treatment structure.

Chapter 4 - Results and Discussion

4.1 Flour Physiochemical Test Data

Figure 4.1 Whole Wheat Flour Granulation Curve



The whole wheat flour granulation data for 8, 50, and 91 day wheat is shown for both RT and FZ storage conditions. The RT and FZ flour granulation was analyzed in triplicate.

The particle size distribution of WWFL is a crucial element that can affect the baking quality of whole wheat flour. Therefore, it was important that the particle size distribution remained similar throughout the study. All the WWFL was combined and blended before particle size analysis for each aging period and storage condition. The WWFL granulation results for 8, 50, and 91 day stored wheat are shown in Figure 4.1 and Table E.2. The data are very similar across wheat age and storage temperature. The feed rates for the first, second, and third passes over the Ross table top mill stand increased over wheat age for both storage temperatures (Table E.1).

Table 4.1 Fisher Sub Sieve Sizer

Average Particle Size (μm)		
Wheat Age (Days)	RT	FZ
8	19.57 ± 1.44	21.00 ± 0.53
50	23.50 ± 1.18	22.90 ± 0.36
91	23.67 ± 0.58	23.21 ± 0.29

Mean Fisher Sub Sieve Sizer particle size (μm) over wheat age (days) ± SD. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). The RT and FZ flour was analyzed in triplicate.

Posner and Deyoe (1986), found that flour particle size changes with wheat age. Specifically, the particle size of flour milled from new crop wheat increased with wheat storage initially but decreased after 19 weeks. As the aging process proceeds, larger flour particles are formed due to a hypothesized increase in adhesion between the starch and protein (Posner and Deyoe, 1986). The Fisher Sub Sieve Sizer data (Table 4.1) shows that in this case WWFL particle size did increase with wheat age as well. The wheat stored at RT and FZ conditions showed particle size increases of 4.1μm and 2.17μm over 91 days of wheat storage respectively. The WWFL particle sizes were similar when compared across storage conditions of the same wheat age.

Table 4.2 Alpine

Mean % Through 75μm		
Wheat Age (Days)	RT	FZ
8	42.00 ± 0.40	36.93 ± 0.83
50	37.33 ± 0.23	37.33 ± 0.46
91	42.00 ± 0.00	42.93 ± 0.23

Mean percent of WWFL that fell through a 75 μm screen over wheat age (days) ± SD. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). The RT and FZ flour was analyzed in triplicate.

The Alpine results for wheat stored at RT showed that the flour particle size increased between the 8 and 50 day wheat study and decreased between the 50 and 91 day aging period

(Table 4.2). The FZ wheat particle size decreased over wheat age when measured using this technique (Table 4.2). The 8 day wheat stored at RT had a smaller particle size distribution than did the 8 day wheat stored at FZ when comparing across storage temperatures. Particle sizes were similar between storage conditions for both the 50 and 91 day wheat studies.

The Fisher Sub Sieve Sizer measured the average particle size by determining the resistance of the flour sample to air flow. The Alpine measured the particle size distribution by determining the percentage of material that passed through the 75μm screen. The Fisher Sub Sieve Sizer showed an increase in flour particle size while the Alpine showed a decrease in particle size over wheat age which seems contradictory. The increase in flour particle size recorded by the Fisher Sub Sieve Sizer would not directly affect the percent throughs of the 75 micron screen used by the Alpine. It is not known if the larger flour particles (around 75μm) increased over wheat age by the same amount as the average sized particles (20-23μm) did (Table 4.1). Even with increases in the average particle size (Table 4.1), those flour particles would still easily pass through the 75 micron screen used for the Alpine.

Table 4.3 Mean WWFL % Moisture and % Protein

	% Moisture		% Protein, 14% m.b.	
Wheat Age (Days)	RT	FZ	RT	FZ
8	10.91 ± 0.05	11.09 ± 0.04	13.91 ± 0.08	13.99 ± 0.14
50	10.79 ± 0.08	11.11 ± 0.09	13.99 ± 0.09	14.08 ± 0.06
91	11.02 ± 0.02	11.05 ± 0.01	14.21 ± 0.14	14.15 ± 0.18

Mean % moisture and % protein content over wheat age (days) for both RT and FZ storage treatments ± SD. The RT and FZ flour was analyzed in triplicate.

Table 4.4 Mean % Wheat Moisture

Wheat Age (Days)	RT	FZ
8	11.30 ± 0.08	11.25 ± 0.01
50	10.86 ± 0.07	11.03 ± 0.10
91	11.17 ± 0.08	10.95 ± 0.05

Change in wheat % moisture over time (days) for both RT and FZ storage treatments ± SD. The RT and FZ flour was analyzed in triplicate.

Not surprisingly, both the WWFL % moisture and % protein contents and wheat % moisture content remained stable over wheat age (Table 4.3; Table 4.4). The falling number values of the unmalted flour and by association the alpha amylase activity stayed constant over wheat age for both the FZ and RT storage conditions (Table 4.5). A falling number value between 250 and 300 seconds was achieved for the malted WWFL for each aging period (Table 4.5). The amount of malt added per 100g of WWFL increased over wheat age for the RT treatment while the FZ treatment had minimal change.

Table 4.5 WWFL Falling Number

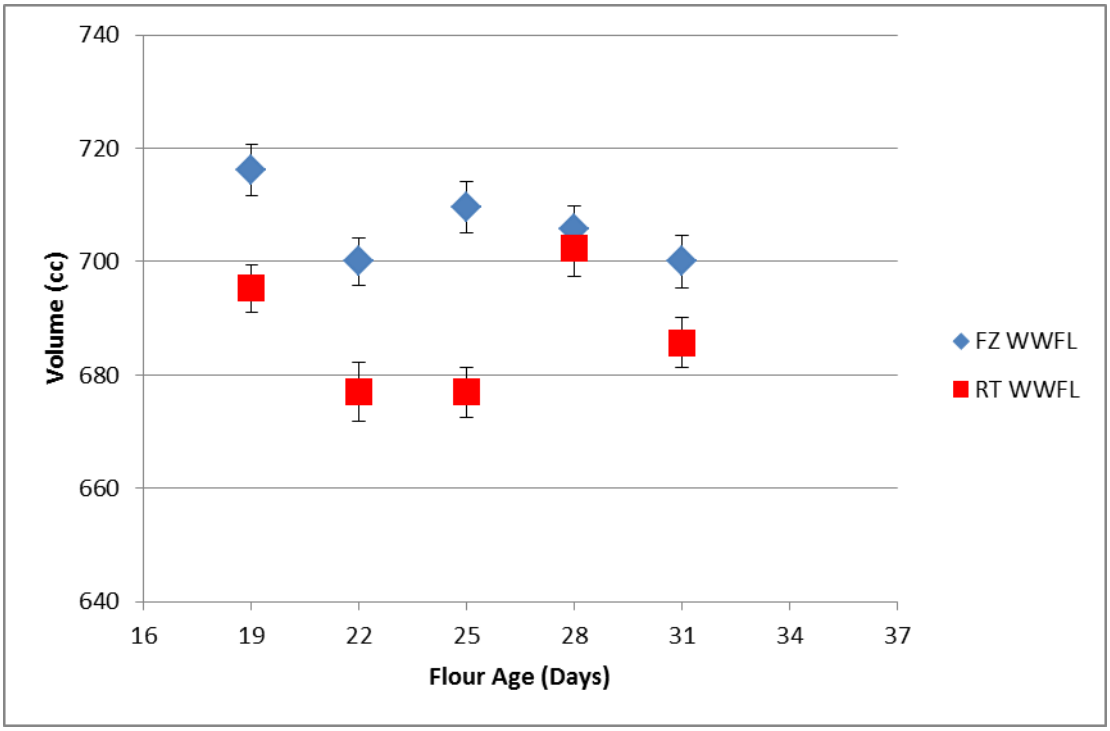
	Malted WWFL (sec)		Unmalted WWFL (sec)		Grams of Malt/100g of WWFL, 14% m.b.	
Wheat Age (Days)	RT	FZ	RT	FZ	RT	FZ
8	252 ± 6.11	253 ± 5.03	418	412	0.671	0.586
50	260 ± 5.13	253 ± 8.50	448 ± 1.41	411 ± 7.78	0.714	0.614
91	258 ± 7.55	257 ± 5.69	439 ± 9.19	416 ± 3.54	0.800	0.614

Mean malted and unmalted WWFL Falling Number values (seconds) over wheat age (days) for both RT and FZ storage treatments ± SD. The mean unmalted WWFL Falling Number values were determined using 1 sample for 8 day wheat and duplicates for 50 and 91 day wheat studies. The Falling Number values of the malted samples were analyzed in triplicate. The grams of malt added per 100g of WWFL at a 14% moisture basis is shown.

4.2 8 Day Wheat Study

4.21 Baking Results

Figure 4.2 Mean FZ 8 Day Wheat Volume vs. Flour Age



Mean 8 day wheat loaf volume (cc) as a function of flour age (days) under FZ (-26˚C) and RT (23˚C) storage conditions ± SE; n~16.

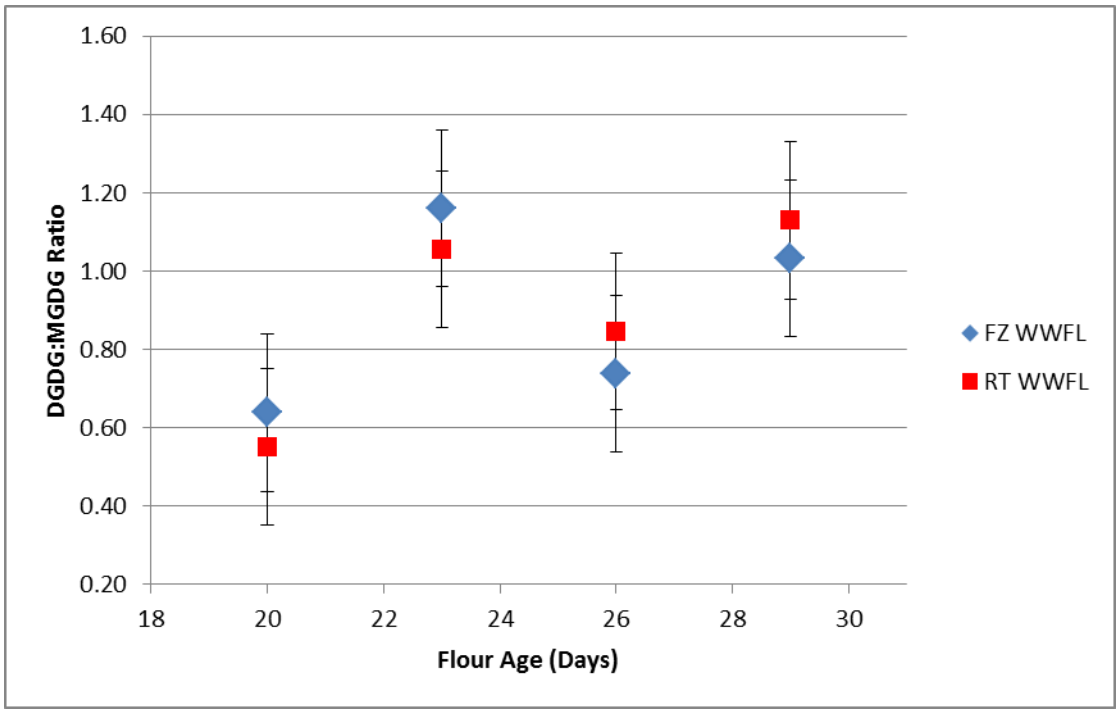
The 8 day new crop wheat mean loaf volumes of bread baked from samples stored at FZ decreased as the flour aged (Figure 4.2). The 8 day wheat mean volumes were unchanged between 22 and 31 days of postmilling storage (Table B.1). The whole wheat flour stored at FZ produced significantly larger mean volumes than did the whole wheat flour stored at RT for every bake except the 28 day flour bake (Table B.1). All the volumes of bread baked from flour stored at FZ were numerically larger than the volumes of bread baked from flour stored at RT (Table B.1).

The 8 day wheat stored at RT did not show a trend in mean volumes throughout flour storage (Figure 4.2). Thus flours baked 22, 25, and 31 days postmilling were all statistically equivalent (Table B.1). The 19 day bake mean volume was significantly larger than the 22 and

25 day bakes (Table B.1). The mean volume peaked at 28 days postmilling (Figure 4.2). The whole wheat bread baked from flour aged 31 days resulted in a decrease in mean volume.

4.22 Glycolipid Results

Figure 4.3 Mean 8 Day Wheat DGDG:MGDG Ratio by Storage Condition over Flour Age



Mean 8 day wheat DGDG:MGDG ratio ± SE; n=3. Storage conditions over flour age: FZ (-26˚C) and RT (23˚C).

The DGDG:MGDG ratios were calculated by dividing the DGDG by the MGDG amounts. The DGDG and MGDG amounts were expressed as the signal (as compared to 1 nmol of standard) per mg of defatted flour. Each DGDG and MGDG level was the sum of the amounts of all the different lipid acyl carbon and double bond configurations.

Under frozen conditions the mean DGDG:MGDG ratios increased over flour age (Figure 4.3; Table C.3). The overall average ratio was 0.89 (Table C.4) so the mean DGDG levels were slightly lower than the mean MGDG levels for the FZ 8 day wheat study. Mean FZ DGDG levels increased over flour age (Table C.1) while the mean MGDG levels remained unchanged

(Table C.2). As the mean DGDG:MGDG ratios increased there were decreases in loaf volume over flour age for the FZ 8 day wheat.

The mean RT DGDG:MGDG ratios were nearly identical to those of the FZ treatment over flour age (Table C.3). The mean ratios increased over flour age (Figure 4.3) with an overall average value of 0.90 (Table C.4). Mean DGDG levels were slightly lower than the mean MGDG levels for the RT 8 day wheat. Again, mean RT DGDG levels increased over flour age (Table C.1) while the mean MGDG levels decreased (Table C.2). While the mean DGDG:MGDG ratios increased, there were minimal changes in mean loaf volume over flour age.

Overall, mean DGDG levels were statistically equivalent over flour age and between storage conditions (Table C.1). The mean MGDG levels were statistically equivalent over flour age and between storage conditions (Table C.2). Finally the mean DGDG:MGDG ratios were statistically equivalent over flour age and between storage conditions with 1 exception, the RT 20 day flour had a significantly smaller ratio than did the 29 day flour (Table C.3).

It appears that there was minimal hydrolysis of a glycosidic bond between 2 of the carbohydrate moieties of DGDG over flour age (which would lead to the creation of more MGDG). Mean MGDG levels were initially higher than DGDG levels for both storage conditions which resulted in DGDG:MGDG ratios less than 1 for the 8 day wheat study.

The mean DGDG:MGDG ratios did not correlate with changes in loaf volume over flour age for both storage treatments. Both the mean volume and DGDG:MGDG ratio values had few significant differences over flour age. The change in the mean DGDG:MGDG ratio over flour age would have changed the polarity of the glycolipid fraction and thereby could possibly have affected gas cell stabilization as described by Sroan et al (2009b). Changes in the mean DGDG:MGDG ratio over flour age and its effects on loaf volume is unknown.

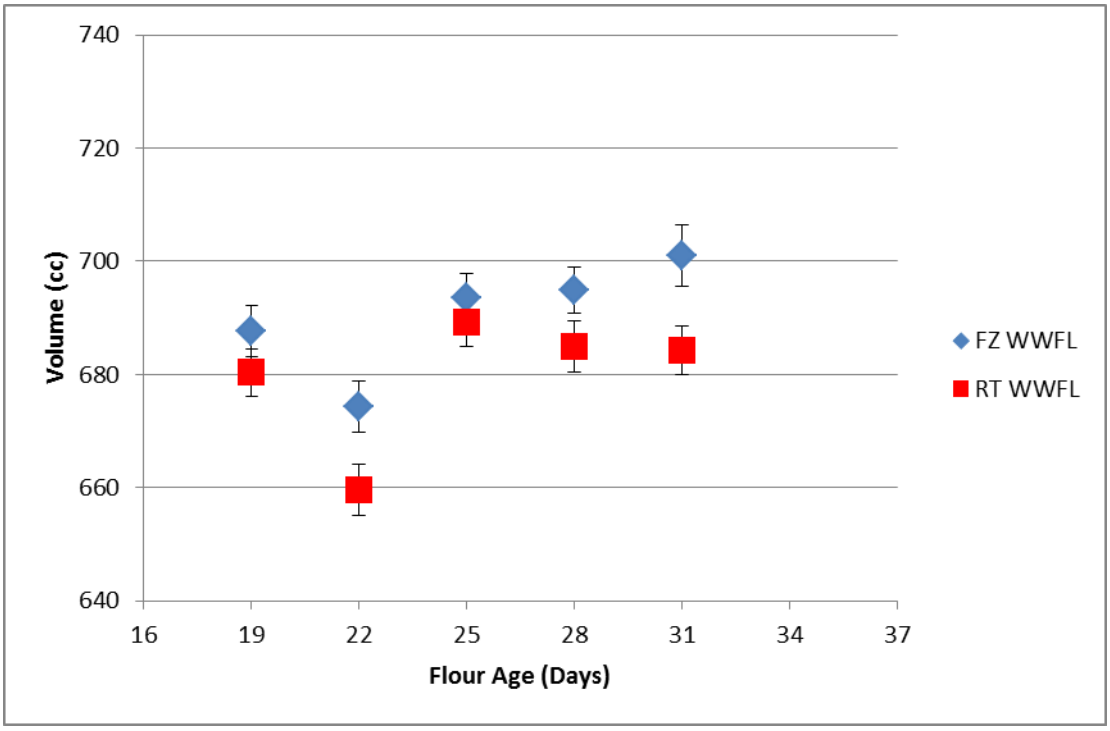
Negligible fatty acid hydrolysis occurred in both the RT and FZ 8 day wheat studies over flour age as shown by the insignificant formation of DGMG and MGMG. Clayton and Morrison (1972) and Warwick et al (1979) found that hydrolysis of MGDG to MGMG and DGDG to DGMG occurred over flour age. Conversely, this study found that negligible hydrolysis of the fatty acids from DGDG and MGDG occurred during both RT and FZ 8 day wheat studies over flour age. This doesn't discount the activity of lipase in the whole wheat flour which could have been acting on triacylglycerols present in the flour.

There was little difference between the RT and FZ glycolipid composition over flour age. It appears that some other factor or change is causing the FZ treatment to produce loaves with higher volumes than the RT storage. The lower storage temperatures could be inhibiting some biochemical change not affecting the glycolipids, leading to higher volumes than the RT storage treatment.

4.3 50 Day Wheat Study

4.31 Baking Results

Figure 4.4 Mean FZ 50 Day Wheat Volume vs. Flour Age



Mean 50 day wheat loaf volume (cc) as a function of flour age (days) under FZ (-26˚C) and RT (23˚C) storage conditions ± SE; n~16.

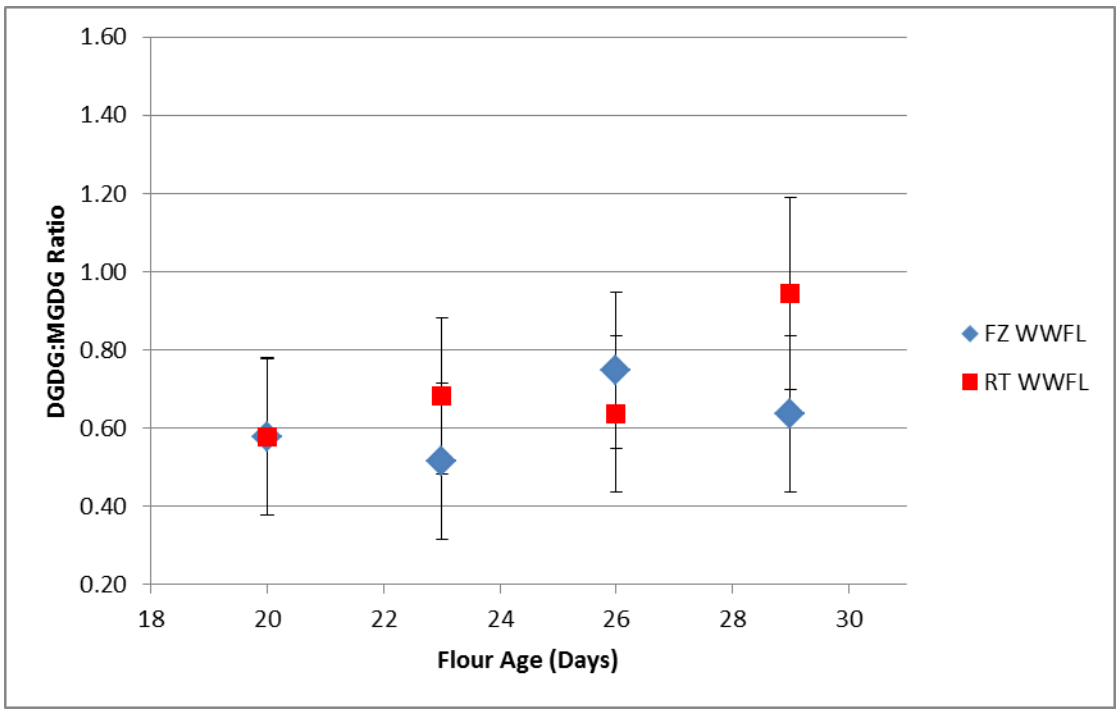
The 50 day new crop wheat mean volume of bread baked from samples stored at FZ had minimal change throughout 31 days of storage (Figure 4.4). Minimal volume change was anticipated at -15˚F due to the probable inhibition of enzymatic reactions of the lipase in the

bran and lipoxygenase found mainly in the germ. 50 day wheat flour aged for 22 days resulted in significantly lower mean loaf volumes than did flour baked after 19, 25, 28, and 31 days of storage (Figure 4.4; Table B.1). There were no other significant differences in mean volume among FZ bakes for 50 day wheat (Table B.1). When comparing mean volumes for 50 day wheat bread baked from samples stored at FZ and RT, FZ flour aged for both 22 and 31 days postmilling were significantly higher than the corresponding RT bakes (Table B.1). All of the other bakes were statistically equivalent between the 2 storage treatments (Table B.1). That said, all of the mean volumes of bread baked from samples stored at FZ were *numerically* larger than those mean volumes of bread baked from RT flour.

The 50 day wheat mean volumes of bread baked from flour stored at RT exhibited minimal change over flour age (Figure 4.4). The flour baked 19, 25, 28, and 31 days after milling were all statistically equivalent (Table B.1). Flour aged for 22 days resulted in a significantly lower mean volume than all the other bakes (Table B.1).

4.32 Glycolipid Results

Figure 4.5 Mean 50 Day Wheat DGDG:MGDG Ratio by Storage Condition over Flour Age



Mean 50 day wheat DGDG:MGDG ratio ± SE; n=3. Storage conditions over flour age: FZ (-26˚C) and RT (23˚C).

The DGDG:MGDG ratios were calculated by dividing the DGDG by the MGDG amounts. The DGDG and MGDG amounts were expressed as the signal (as compared to 1 nmol of standard) per mg of defatted flour. Each DGDG and MGDG level was the sum of the amounts of all the different lipid acyl carbon and double bond configurations.

Under freezing conditions, the mean DGDG:MGDG ratios increased over flour age (Figure 4.5; Table C.3). The overall average ratio was 0.62 (Table C.4) so the mean DGDG levels were lower than the mean MGDG levels throughout the FZ 50 day wheat study. The mean FZ DGDG and MGDG levels decreased slightly over flour age. As the mean FZ DGDG:MGDG ratios increased there were minimal changes in loaf volume over flour age. The change in the mean FZ DGDG:MGDG ratios did not correlate to changes in mean loaf volume.

At RT the mean DGDG:MGDG ratios increased over flour age (Figure 4.5; Table C.3) with the overall average at 0.71 (Table C.4). Again, the mean RT DGDG levels were lower than

the mean MGDG levels for the RT 50 day wheat study. The mean RT DGDG levels increased over flour age while the mean MGDG levels decreased. The increasing mean RT DGDG:MGDG ratios did not correlate with changes in volume over RT storage.

The mean DGDG levels were statistically equivalent over flour age and between storage conditions (Table C.1). The same was true for mean MGDG levels over flour age and between storage conditions (Table C.2). Therefore the mean DGDG:MGDG ratios were statistically equivalent over flour age and between storage conditions (Table C.3).

It appears that there was minimal hydrolysis of a glycosidic bond between 2 of the carbohydrate moieties of DGDG over flour age (which would lead to the creation of more MGDG). Mean MGDG levels were initially higher than mean DGDG levels for both storage conditions which resulted in DGDG:MGDG ratios less than 1.

As was true for the 8 day wheat, the mean DGDG:MGDG ratios did not correlate with changes in mean loaf volume over flour age for both storage treatments. Both the mean volume and DGDG:MGDG ratio values had few significant differences over flour age. The change in the mean DGDG:MGDG ratio over flour age changes the polarity of the glycolipid fraction and therefore could affect gas cell stabilization as described by Sroan et al (2009b). Changes in the mean DGDG:MGDG ratio over flour age and its effects on loaf volume is unknown.

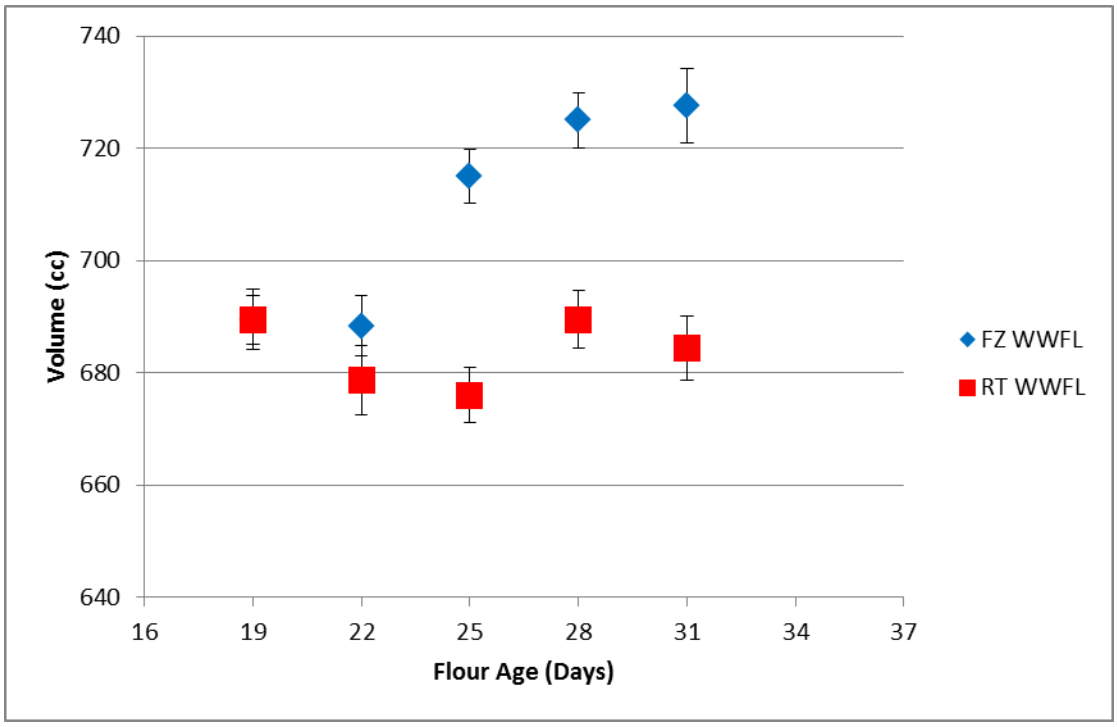
Negligible fatty acid hydrolysis occurred in the both the RT and FZ 50 day wheat studies over flour age as shown by the insignificant formation of DGMG and MGMG. This doesn't discount the activity of lipase in the whole wheat flour which could have been acting on triacylglycerols present in the flour.

There was little difference between the RT and FZ glycolipid composition over flour age. It appears that some other factor or change is causing the FZ treatment to produce loaves with higher volumes than the RT storage. The lower storage temperatures could be inhibiting some biochemical change not affecting the glycolipids, leading to higher volumes than the RT storage treatment.

4.4 91 Day Wheat Study

4.41 Baking Results

Figure 4.6 Mean FZ 91 Day Wheat Volume vs. Flour Age



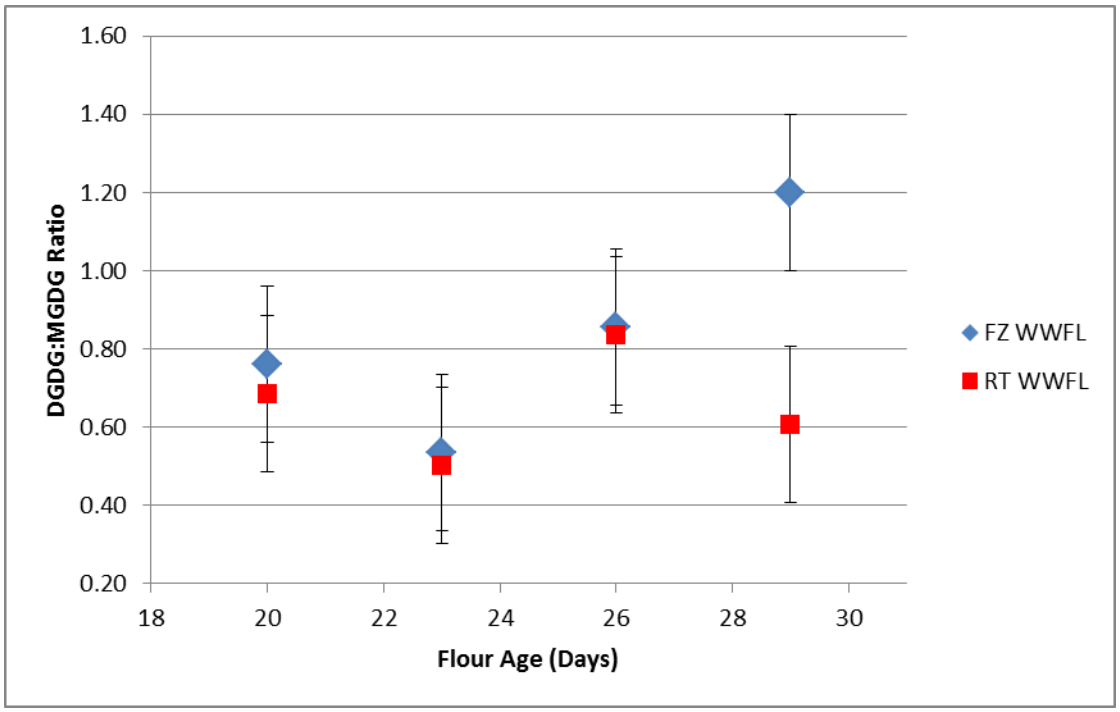
Mean 91 day wheat loaf volume (cc) as a function of flour age (days) under FZ (-26˚C) and RT (23˚C) storage conditions ± SE; n~16.

The FZ 91 day wheat mean loaf volumes of bread baked from samples stored at FZ increased as the flour aged (Figure 4.6). The flours aged for 19 and 22 days had significantly lower mean volumes than did flours baked 25, 28, and 31 days postmilling (Table B.1). The mean volumes of flour baked 25, 28 and 31 days postmilling were statistically equivalent (Table B.1). When comparing mean volumes of bread baked from samples stored at FZ and RT from 91 day aged wheat, the FZ flour aged 25, 28, and 31 days had significantly higher mean volumes than the corresponding RT bakes (Table B.1). FZ flour baked 22, 25, 28, and 31 days postmilling had only numerically higher mean volumes than did the RT flours (Table B.1).

Similar to the RT 8 and 50 day wheat studies, the mean 91 day wheat volumes of bread baked from samples stored at RT had minimal change over flour age (Figure 4.6). All the mean volumes were statistically equivalent over flour age (Table B.1).

4.42 Glycolipid Results

Figure 4.7 Mean 91 Day Wheat DGDG:MGDG Ratio by Storage Condition over Flour Age



Mean 91 day wheat DGDG:MGDG ratio ± SE; n=3. Storage conditions over flour age: FZ (-26˚C) and RT (23˚C).

The DGDG:MGDG ratios were calculated by dividing the DGDG by the MGDG amounts. The DGDG and MGDG amounts were expressed as the signal (as compared to 1 nmol of standard) per mg of defatted flour. Each DGDG and MGDG level was the sum of the amounts of all the different lipid acyl carbon and double bond configurations.

The mean DGDG:MGDG ratios of the 91 day wheat increased over flour age (Figure 4.7; Table C.3). The overall average ratio was 0.84 (Table C.4) so the mean DGDG levels were lower than the mean MGDG levels for the FZ 91 day wheat study. Mean FZ DGDG levels increased over flour age (Table C.1). The 20 and 23 day flour mean FZ DGDG levels were both

significantly lower than 26 day flour and the 23 day flour was significantly lower than the 29 day value (Table C.1). The mean MGDG levels decreased over flour age except for an increase for the 26 day flour amounts which were significantly higher than the 23 day flour level (Table C.2). It is unclear why the 26 day flour extraction had such high glycolipid levels. Overall, as the mean FZ DGDG:MGDG ratios increased they were correlated with increases in loaf volume over flour age.

The RT 91 day mean DGDG:MGDG ratios were similar to those of the FZ treatment for the first 3 extractions (Figure 4.7). Again it's unclear what lead to the higher glycolipid levels for the 26 day flour. The ratios were variable over flour age with the overall average ratio at 0.66 (Table C.4). The average RT DGDG levels were lower than the average MGDG levels for the RT 91 day wheat study. The mean RT DGDG levels increased over flour age while the mean MGDG levels were unchanged. The 23 day flour RT mean DGDG level was significantly lower than the 26 day flour (Table C.1). The mean RT MGDG values were statistically equivalent over flour age (Table C.2). The variable mean RT DGDG:MGDG ratios were not correlated with changes in volume over RT storage.

There were no significant differences between the mean FZ and RT DGDG levels for the 91 day wheat study (Table C.1). There was 1 significant storage interaction between the FZ and RT MGDG levels which was with the 26 day flour extraction (Table C.2). The mean DGDG:MGDG ratios were similar over flour age and between storage conditions (Table C.3). The mean FZ DGDG:MGDG 23 day ratio was significantly lower than the 29 day ratio (Table C.3). The mean RT ratio had no significant differences within flour age (Table C.3). There was only 1 significant storage interaction which was between the 29 day flours (Table C.3).

There was minimal cleavage of a glycosidic bond between the 2 carbohydrate moieties of DGDG over flour age which would lead to the creation of more MGDG. The mean MGDG levels were initially higher than mean DGDG levels for both storage conditions which resulted in mean DGDG:MGDG ratios less than 1.

The mean FZ DGDG:MGDG ratios were directly correlated with an increase in mean volumes over flour age. At RT the mean DGDG:MGDG ratios were statistically equivalent over flour age and were not correlated to any changes in mean volume for the 91 day wheat study. The 91 day wheat ratios correlated more with changes in volume than it did for the other wheat age studies. The change in the mean DGDG:MGDG ratio over flour age would have changed

the polarity of the glycolipid fraction and thereby could possibly have affected gas cell stabilization as described by Sroan et al (2009b). Changes in the mean DGDG:MGDG ratio over flour age and its effects on loaf volume are unknown.

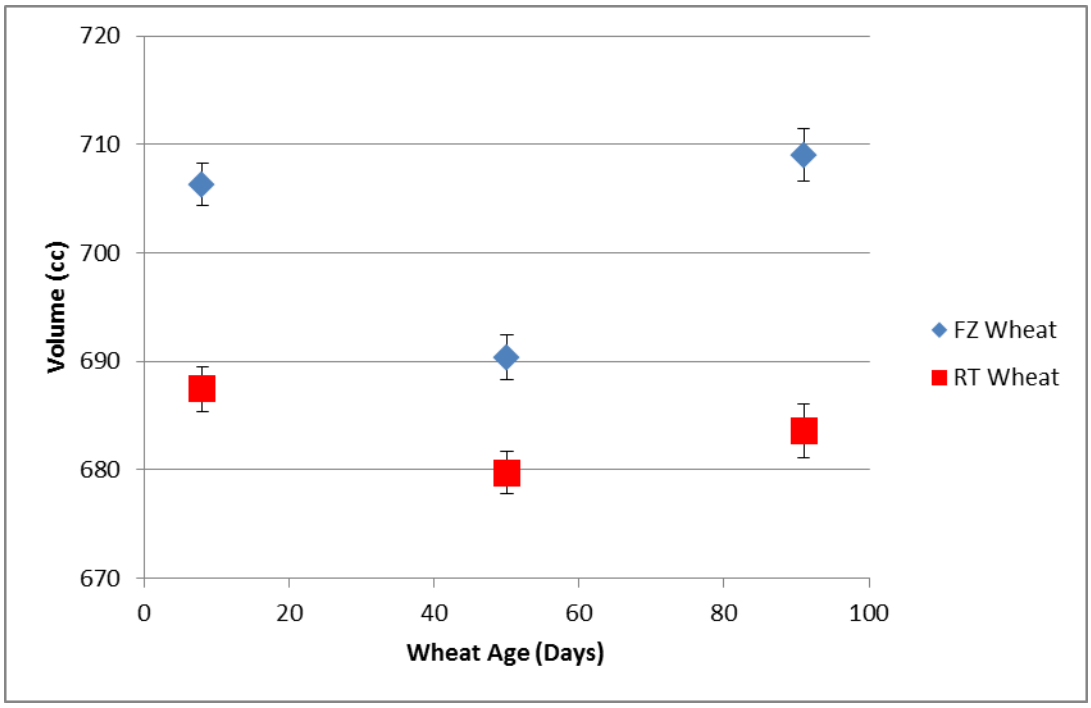
Negligible fatty acid hydrolysis occurred in the both the RT and FZ 91 day wheat studies over flour age as shown by the insignificant formation of DGMG and MGMG. This doesn't discount the activity of lipase in the whole wheat flour which could have been acting on triacylglycerols present in the flour.

There was little difference between the RT and FZ glycolipid composition over flour age. Once again it appears that some other factor or change is causing the FZ treatment to produce loaves with higher volumes than the RT storage. The lower storage temperatures could be slowing down some other reaction occurring in the flour that is detrimental to loaf volume.

4.5 Wheat Age Study

4.51 Baking Results

Figure 4.8 Mean Volume by Storage Condition over Wheat Age



Mean loaf volume (cc) of all 5 bakes within a wheat age study (8, 50, 91 days) ± SE. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C).

When comparing wheat of the same age, wheat stored at -26˚C (FZ) had significantly higher mean volumes than did the wheat stored at 23˚C (RT) (Figure 4.8).

The following description describes changes in mean volume in FZ wheat over wheat age (Figure 4.8; Table B.2; Table B.3). The volume dropped significantly from 706cc to 690cc as the wheat aged from 8 days to 50 days after harvest (Figure 4.8; Table B.3). After 91 days the volume peaked increasing to 709cc. The increase in volume from 690cc to 709cc was significant (Figure 4.8; Table B.3). This agrees with Shellenberger (1939) who found that baking quality was best at a wheat age of 3.5 months. The mean volumes of 8 day and 91 day wheat were exactly the same.

The drop in mean volume for 50 day wheat could have been due to larger final bread weights which lowered mean volume values (This reason is analyzed in detail in Appendix D),

the necessity of using a different brand of yeast for bake 2, and variable temperature conditions in the oven and room.

Wheat stored in RT conditions displayed the trends in volume as wheat stored in FZ conditions (Figure 4.8). There was a significant drop of 7cc between 8 and 50 day wheat mean loaf volumes of bread baked from samples stored at RT (Table B.3). The reasons for this decrease could be the same as those for the drop at FZ conditions (Analyzed in Appendix D). The subsequent increase of 4cc between 50 and 91 day wheat was not significant (Table B.3). The difference in volume between 8 and 91 day old wheat was not significant (Table B.3).

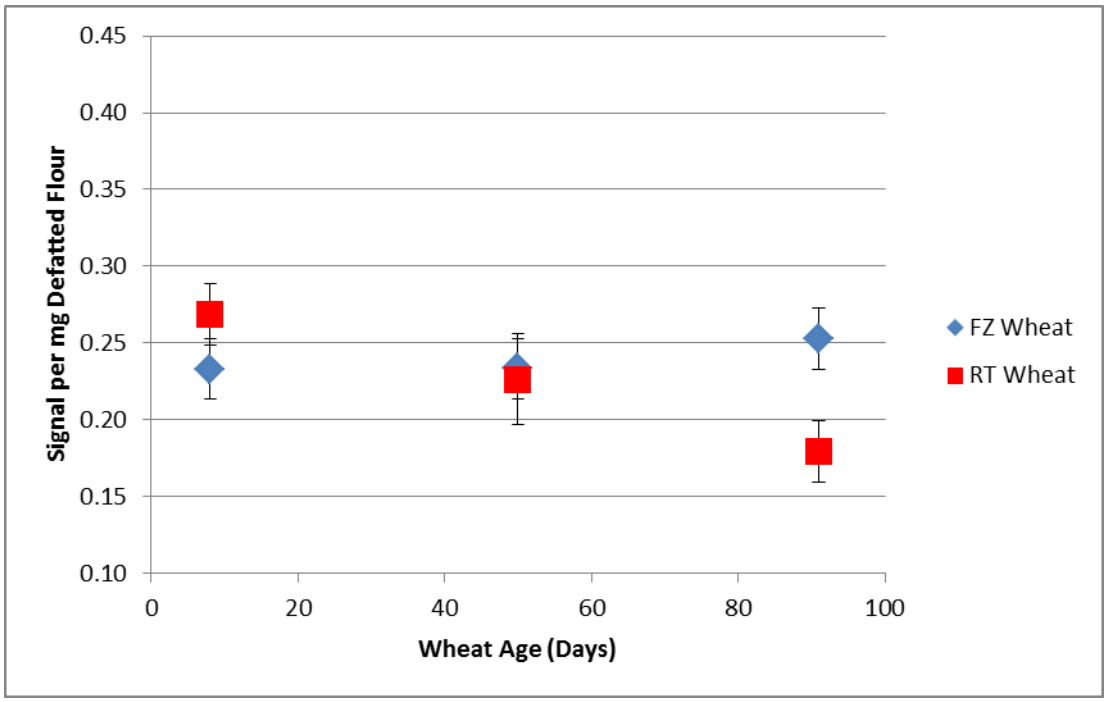
The average FZ volumes for 8, 50, and 91 day wheat were all significantly larger than the RT bakes (Table B.3). The results were not expected since the new crop wheat maturation process is expected to be accelerated at RT storage thus promoting the improved loaf volume of freshly milled flour. It was expected for the aging process to take longer if the wheat is stored under cold temperatures. It appears that the FZ storage conditions may be preventing changes from occurring in either the wheat, flour or both that contribute to lower loaf volumes for RT flour.

4.52 Glycolipid Results

These studies found that negligible hydrolysis of the fatty acids from DGDG and MGDG levels occurred over wheat age contrary to the results of Clayton and Morrison (1972) and Warwick et al (1979). The amounts and accumulation of mean MGMG and DGMG levels present in the whole wheat flour over wheat age was negligible.

The DGDG and MGDG lipid species were the most common in the whole wheat flour. The most common lipid acyl carbon and double-bond configuration for both MGDG and DGDG was 36:4 which agrees with Finnie et al (2009). The fatty acid configuration was most likely 18:2/18:2.

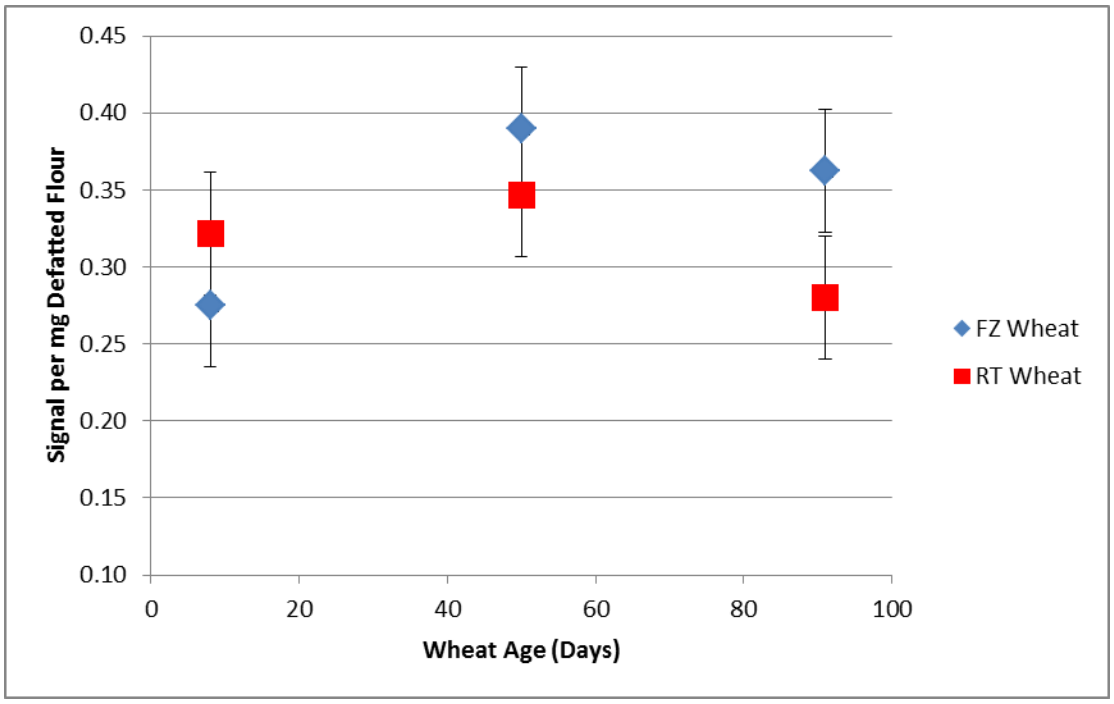
Figure 4.9 Mean DGDG Levels by Storage Condition over Wheat Age



Mean DGDG level (signal per mg of defatted flour extracted from bound lipids) of all 4 extractions within a wheat age study (8, 50, 91 days) ± SE. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). DGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts.

There was a decrease in the amount of mean RT DGDG over wheat age (Figure 4.9). The decrease in mean RT DGDG levels occurred incrementally over wheat age which resulted in a significant difference between the 8 and 91 day wheat studies (Table C.5). Mean FZ wheat DGDG levels increased slightly over wheat age (Figure 4.9; Table C.5). After 91 days of wheat storage, FZ wheat had significantly higher mean DGDG levels than RT stored wheat (Table C.5). The difference between FZ and RT mean DGDG levels did not appear to have any effects on loaf volume. Under frozen conditions, FZ treatment (8, 50, and 91 day) wheat all had larger mean volumes than the RT storage. Differences in mean volume (between storage conditions) occurred when the both the FZ and RT mean DGDG levels were statistically equivalent for 8 day wheat and significantly *different* for the 91 day wheat. The significant drop in mean RT DGDG levels doesn't correlate with any difference in volume between RT 8 and 91 day wheat studies.

Figure 4.10 Mean MGDG Levels by Storage Condition over Wheat Age

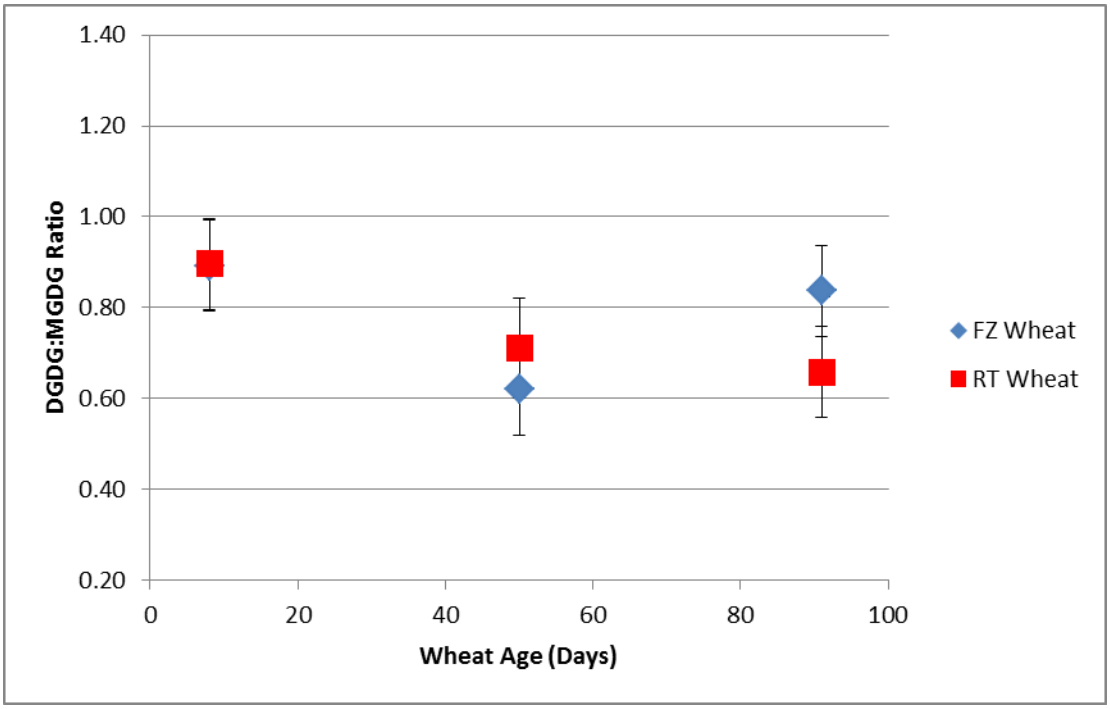


Mean MGDG level (signal per mg of defatted flour extracted from bound lipids) of all 4 extractions within a wheat age study (8, 50, 91 days) ± SE. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). MGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts.

The mean MGDG levels increased between the 8 and 50 day wheat studies and decreased between the 50 and 91 day wheat studies for both storage temperatures (Figure 4.10; Table C.6). Those values were statistically equivalent over wheat age and between storage conditions (Table C.6). The reason for the initial increase and eventual decrease in mean MGDG levels is unknown. It's expected that an increase in MGDG would be due to a glycosidic bond cleavage and result in a decrease in DGDG levels. The mean FZ DGDG amounts were unchanged which did not correlate with the increase in mean MGDG levels. The mean RT DGDG amounts decreased over time which correlated with the increase in mean MGDG amounts between the 50 and 91 day wheat. There appeared to be some glycosidic bonds cleaved between the carbohydrate moieties of DGDG over wheat age for the RT treatment which created more MGDG. Negligible hydrolysis of DGDG glycosidic bonds occurred over wheat age for the FZ treatment.

This study confirmed that mean DGDG and MGDG levels were higher for FZ wheat than for RT stored wheat (except for 8 day wheat) which correlated with the findings of Daftary and Pomeranz (1965) that the polar lipid content was greater for wheat stored at 4˚C after 140 days (0.67%, HRW and 0.77%, SRW) than storage at 49˚C (0.38%, HRW and 0.39%, SRW).

Figure 4.11 Mean DGDG:MGDG Ratio Levels by Storage Condition over Wheat Age



Mean DGDG:MGDG ratio of all 4 extractions within a wheat age study (8, 50, 91 days) ± SE. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C).

The DGDG:MGDG ratios were calculated by dividing the DGDG by the MGDG amounts. The DGDG and MGDG amounts were expressed as the signal (as compared to 1 nmol of standard) per mg of defatted flour. Each DGDG and MGDG level was the sum of the amounts of all the different lipid acyl carbon and double bond configurations.

This study also found that the mean DGDG:MGDG ratios decreased over wheat age (Figure 4.11). The ratios decreased the most between 8 day and day 50 wheat studies for both storage temperatures (Figure 4.11, Table C.4) and was apparently due to the sharp increase in mean MGDG amounts between the same storage periods. The ratios actually increased between the 50 day and 91 day wheat studies for FZ wheat. Overall, the mean DGDG:MGDG ratios were statistically equivalent over wheat age and between storage conditions (Table C.4).

As pointed out in the baking results section, mean volumes initially decreased between the 8 and 50 day wheat studies and then increased as the wheat aged up to 91 days. At first glance, the decrease in mean volume between 8 and 50 day storage studies could be related with the decrease in the mean DGDG:MGDG ratios between the same wheat aging periods for both storage conditions. The increase in mean volume between the RT 50 and 91 day wheat study was harder to explain based on the mean DGDG:MGDG ratios because the RT ratios continued to decrease between the 50 and 91 day RT wheat. The RT 8 and 91 day wheat mean volumes for both storage treatments were statistically equivalent but a sizable difference existed between the RT 8 and 91 day wheat mean DGDG:MGDG ratios. The FZ ratio increased substantially between the 50 and 91 days of storage which mimicked the FZ mean volume increase between the same periods. It is still unclear how changes in the polarity of the glycolipid fraction affected volumes in these cases.

It did not appear that changes in the glycolipid fraction had any effect on the volume for both the RT and FZ treatments over wheat age. It is speculated that there are 1 or more changes occurring at RT treatment that are slowed down in the FZ storage (and that this results in higher mean volumes for the FZ storage).

Chapter 5 - Conclusions

5.1 Baking Study

The change in loaf mean volume of bread baked from samples stored at RT was minimal over flour age for the 8, 50, and 91 day wheat studies (Figures 4.2; 4.4; and 4.6). The results for RT stored WWFL were not expected. Based on previous work, there were 2 possible outcomes. One was that baking quality would improve as the flour aged (Posner and Deyoe 1986; Shelke et al 1992a; Chen and Schofield 1996) as seen with refined flour. Posner and Deyoe (1986) and Chen and Schofield (1996) found that freshly milled hard wheat flour showed an improvement in loaf volume during storage close to room temperature. Chen and Schofield (1996) recorded increases in loaf volume as soon as 10 days after milling. While Posner and Deyoe (1986) did not report significant loaf volume changes in 3 weeks of flour storage using new crop wheat from the 1983 crop year, it took 126 days of aging to achieve the peak volume in 1984. The second prediction would be that WWFL loaf volume would decrease throughout storage as reported by Barnes and Lowy (1986). Who found that loaf volumes for WWFL stored at 20˚C decreased at a faster rate than did WWFL stored at -20˚C (Barnes and Lowy 1986).

The change in loaf mean volume of bread baked from flour stored at RT was minimal over wheat age except for the significant drop in volume between the 8 and 50 day wheat studies. This was unexpected due to the improvement in baking quality over wheat age as seen in previous work (Shellenberger 1939; Posner and Deyoe 1986; Shelke et al 1992a).

Here the FZ treatment resulted in mean volumes significantly larger than the RT treatment over both wheat and WWFL age (Tables B.1; B.3). The FZ 8 day wheat study showed a decrease in loaf mean volume over WWFL age. The 50 day wheat mean volumes stayed constant and the 91 day wheat showed an increase over WWFL age. The WWFL loaf volume results under FZ conditions were unexpected. If the reactions were biochemical in nature, one would expect that FZ storage would slow down those reactions (prolonging the flour maturing process) producing smaller loaf volumes than the RT treatment. The colder storage temperatures might be inhibiting some biochemical process that results in the FZ WWFL producing larger loaf volumes than the RT treatment. The results from this study are consistent with Barnes and Lowy (1986) and Galliard and Collins (1988) where lower loaf volumes were observed for wholemeal

flour stored at +20˚C than at -20˚C. Interestingly, Yoneyama et al (1970) found that flour maturation occurred at a faster rate at 30˚C than storage at -30˚C and 0˚C over 60 days but 30˚C storage still produced loaves with lower volumes.

The changes at FZ conditions in loaf mean volume were minimal over wheat age (except for the 50 day wheat study). The FZ conditions also resulted in mean volumes larger than the RT condition. Minimal change in loaf mean volume was expected over wheat age due to the colder storage conditions slowing down biochemical processes. It was unexpected that the FZ condition resulted in mean volumes larger than the RT condition over wheat age for reasons already discussed for the WWFL.

The hypothesis for the baking studies was that the wheat and WWFL baking quality would change over time. The room temperature storage condition would increase the rate of change while the freezing conditions would slow down change over time. The results from this research did not validate these hypotheses. One possible explanation for the minimal change in loaf mean volume over wheat and flour age was that the baking method proofed the dough to height and not time. This could have affectively minimized differences in volume that otherwise, would have been apparent.

FZ storage of wheat or flour immediately resulted in loaves with larger mean volumes than RT treatment. What was causing this difference in mean volume or what reaction or enzyme does lower storage temperatures possibly slow down remains to be determined. It is possible that whatever the FZ condition is inhibiting is preventing change from happening in the lipid composition which appears to play a role in reducing the baking quality of wholemeal flour (Galliard 1986b).

The lipid results indicated that negligible hydrolysis of DGDG to DGMG and MGDG to MGMG occurred for under both FZ and RT conditions. This could be due to limited lipase activity. Lipase is most active at 17% moisture and only 50% active between 10 and 14% moisture (Doblado-Maldonado et al 2012). The average RT and FZ wheat (11.11%, RT; 11.08%, FZ) and flour (10.91%, RT; 11.08%, FZ) moisture would indicate that lipase activity was retarded. There might not have been enough moisture in the wheat and flour to allow the lipase to be distributed through the samples. Free fatty acid formation isn't always correlated with a decrease in baking quality so other reactions could be expected to contribute to variable flour baking quality (Clayton and Morrison 1972). If the lipase activity was limited,

lipoxygenase might be responsible for lower loaf volumes at RT storage. Lipoxygenases activity is higher at lower moisture contents as found in this experiment (Maraschin et al 2008). After 16 weeks of storage at 22˚C refined flour at 10% moisture only lost 2.4% of its lipoxygenase activity compared to a 34% loss for 14% moisture flour (Maraschin et al 2008). Wholemeal flour would be expected to contain more nonpolar triacylglycerols that have more linoleic fatty acids available for lipoxygenase activity (Chung et al 2009). Upon hydration lipoxygenase, present primarily in the germ, oxidizes polyunsaturated fatty acids and to a lesser degree fatty acids present in tri, di, and monoacylglycerols (Galliard 1986a; Tait and Galliard 1988). Linoleic acid is the main PUFA present in triacylglycerols and therefore the main target of wheat lipoxygenase (Tait and Galliard 1988; Hidalgo and Brandolini 2012). Lipoxygenase converts free fatty acids into lipid hydroperoxides which are enzymatically broken down into monohydroxy-octadecadienoic acid methyl ester (MHA) and dihydroxy-octadecenoic acid methyl ester (DHA) (Warwick and Shearer 1980). The reduction of hydroperoxides to hydroxy acids may affect dough rheology by oxidizing sulfhydryl groups into disulfide bonds (Chung et al 1978; Shiiba et al 1991). It is unknown how hydroxy acids impact baking quality (Tait and Galliard 1988). Because hydration of the flour is necessary for the acceleration of lipoxygenase activity, the lipid extraction/analysis procedure used in this study may not have detected all of the changes in lipid composition due to this enzyme. The inhibition of lipoxygenase by the FZ treatment could have affected the flour's baking quality allowing for higher volumes than the RT treatment.

Another reason for negligible hydrolysis of glycolipid fatty acids could be that the bran lipase preferably acts on triacylglycerols (Pyler 1988; Arpigny and Jaeger 1999). Consequently, whole wheat flour contains an abundance of neutral lipids (and therefore triacylglycerols) due to the presence of germ. The lipase could have enough triacylglycerols to act on and therefore have no need to act on the glycolipid fatty acids. The increase in free polyunsaturated fatty acids could result in higher lipoxygenase activity which would cause a shift in lipid composition upon flour hydration thereby effecting loaf volume. Polyunsaturated fatty acids (PUFA) can reduce the amount of lipid binding to the gluten proteins during mixing which hinders the gas retaining properties of the lipoprotein complex in wheat flour doughs (Doblado-Maldonado et al 2012). The FZ treatment might have inhibited the hydrolysis fatty acids from triacylglycerols and their oxidation resulting in higher than the RT treatment.

5.2 Glycolipid Study

Changes to the glycolipid fraction during storage could affect bread quality (Hoseney et al 1970). To determine the possible effect of mean DGDG:MGDG ratio change on loaf volume, determining how the individual DGDG and MGDG glycolipid species affect loaf volume as well as how changes to the polarity of the glycolipid fraction affect bread quality is important.

Selmair and Koehler (2009) found that DGDG purified from soybean lecithin increased loaf volume more than did MGDG. The loaf volume improvement imparted by different glycolipids appeared to vary depending on the ratio of hydrophilic monosaccharides and lipophilic fatty acids present (Selmair and Koehler 2010). Similarly, Pomeranz et al (1969) concluded that loaf volumes differed based on the HLB values of sucroglycerides added into a bread formula. A hydrophilic-lipophilic balance value for interaction at the liquid lamellae-gas cell interface may be necessary for optimum baking potential (Selmair and Koehler 2010). DGDG's have a hydrophilic-lipophilic balance (HLB) between 9.9-10.5 (varying based on fatty acid structure) which is within the optimum HLB range of 8-12 common for oil in water surfactants (Selmair and Koehler 2009). Daftary et al (1968) found that free polar lipids (containing higher amounts of MGDG) provided more loaf volume improvement than did bound polar lipids (containing higher amounts of DGDG), nonpolar lipids, and total free lipids when incorporated into original and defatted flours. The MGDG lipids have an important role in increasing baking potential too. A change in the polarity of the lipid fraction over wheat and flour age could change how effectively the air cells are stabilized during proofing and oven spring.

It was anticipated that flour glycolipid composition would change over wheat and WWFL age. The RT treatment was expected to promote these changes while the FZ conditions would be expected to slow them down. It was anticipated that changes in the glycolipid fraction would reflect changes in the baking quality. The results from this research did not validate these hypotheses.

This study found that changes in the mean DGDG:MGDG ratio did not directly result with changes in loaf volume over both flour and wheat age (except for FZ 91 day wheat). The DGDG and MGDG amounts in the 8 and 50 day wheat studies were nearly all statistically equivalent over both wheat and flour age and between storage conditions. These limited changes corresponded with volumes that were generally statistically equivalent over the same period.

The RT 91 day wheat also showed little change in mean volume and mean DGDG and MGDG amounts over flour age. The FZ 91 day wheat study had the most unpredictable changes in mean DGDG and MGDG amounts over flour age which corresponded with an increase in mean loaf volume over the same period. The increase in mean volume correlated with an increase in the mean DGDG:MGDG ratio. It is unknown how changes in the glycolipid fraction affected loaf volume over both wheat and flour age.

The mean DGDG and MGDG amounts were generally statistically equivalent between storage conditions but the volumes were significantly different over the same aging period between storage conditions. There was negligible hydrolysis of the DGDG and MGDG fatty acids to form DGMG and MGMG respectively.

The mean FZ DGDG amounts were unchanged which did not correlate with the increase in mean MGDG levels. The mean RT DGDG amounts decreased over time which did correlate with the increase in mean MGDG amounts between the 50 and 91 day wheat. There appeared to be some glycosidic bonds cleaved between the carbohydrate moieties of DGDG over wheat age for the RT treatment which created more MGDG. Negligible hydrolysis of DGDG glycosidic bonds occurred over wheat age for the FZ treatment.

Something other than the glycolipids was being affected over wheat and flour age. It is anticipated that some other biochemical change is being inhibited by the cold storage conditions that is affecting whole wheat bread volume. Some possible changes are discussed above in the baking conclusion section.

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Appendix A - Solutions, Solvents and Abbreviations

Solutions

1. Hexane, ethyl acetate, acidic acid (95:5:0.2)
2. Hexane and ethyl acetate (95:5)
3. Chloroform and Methanol (2:1)
4. Tetrahydrofuran acetonitrile, propan-ol-2 (35:35:30)
5. Acetonitrile and methanol (35:65)
6. Isopropanol, water (90:10)
7. Hexane

Solvents: Purchased from Fischer Scientific

Acetic Acid, 500ml, AC42322-5000, Reagent ACS

Acetonitrile, 1L, A21-1, 1L, Certified ACS

Chloroform, 500ml, C298-500, Certified ACS

Chloroform , Acros Organics, 1L, 268320010, HPLC

Ethyl Acetate, 500ml, E145-500, Certified ACS

Hexanes, 4L, H292-4 Certified ACS

Methanol, 1L, A412-1, Certified ACS

2-Propanol, 4L, A417-4, Certified

Tetrahydrofuran, 500ml, T397-500, Certified

Abbreviations:

ASG, acylated steryl glycosides; DAG, diacylglycerols; DGDG, digalactosyldiacylglycerols; DGMG, digalactosylmonoacylglycerols; FA, fatty acids; FFA, free fatty acids; FZ, freezing; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; MAG, monoacylglycerols; MGDG, monogalactosyldiacylglycerols; MGMG, monogalactosylmonoacylglycerols; NAPE, N-acylphosphatidyethanolamine; PA, phosphatidic acid; PC, phosphatdylcholine; RT, room temperature; TAG, triacylglycerols; WWFL, whole wheat flour.

Appendix B - Loaf Volume Data

Table B.1 Mean Loaf Volumes by Storage Condition over Wheat and Flour Age

	8 Day Wheat		50 Day Wheat		91 Day Wheat	
Flour Age (Days)	FZ	RT	FZ	RT	FZ	RT
19	716a ± 4.51	695cb ± 4.20	688b ± 4.51	680bc ± 4.20	689a ± 4.35	689ac ± 5.42
22	700b ± 4.20	677d ± 5.15	674c ± 4.51	660a ± 4.51	688a ± 5.42	679ac ± 6.15
25	710ab ± 4.51	677d ± 4.51	694b ± 4.35	689bc ± 4.35	715b ± 4.70	676c ± 4.91
28	706ab ± 4.20	702ab ±4.91	695b ± 4.07	685bc ± 4.51	725b ± 4.91	690c ± 5.15
31	700b ± 4.70	686cd ± 4.35	701b ± 5.42	684c ± 4.35	728b ± 6.64	684c ± 5.75

Mean whole wheat loaf volume (cc) ± SE; n~16. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). Mean values followed by the same letter are not significantly different at P = 0.05. Only comparisons within flour age and between storage conditions at a constant wheat and flour age are depicted.

Table B.2 Mean FZ and RT Loaf Volumes

	FZ Wheat			RT Wheat		
Flour Age (Days)	8	50	91	8	50	91
19	716a ± 4.51	688b ± 4.51	689b ± 4.35	695a ± 4.20	680b ± 4.20	689ab ± 5.42
22	700a ± 4.20	674b ± 4.51	688a ± 5.42	677a ± 5.15	660b ± 4.51	679a ± 6.15
25	710a ± 4.51	694b ± 4.35	715a ± 4.70	677a ± 4.51	689b ± 4.35	676a ± 4.91
28	706a ± 4.20	695a ± 4.07	725b ± 4.91	702a ± 4.91	685b ± 4.51	690ab ± 5.15
31	700a ± 4.70	701a ± 5.42	728b ± 6.64	686a ± 4.35	684a ± 4.35	684a ± 5.75

Mean whole wheat loaf volume (cc) ± SE; n~16. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). Mean values followed by the same letter are not significantly different at P = 0.05. Only comparisons within storage conditions at a constant flour age are depicted.

Table B.3 Mean FZ and RT Loaf Volumes

Wheat Age (Days)	FZ	RT
8	706a ± 1.98	687b ± 2.07
50	690b ± 2.06	680c ± 1.96
91	709a ± 2.46	684bc ± 2.46

Mean whole wheat loaf volume (cc) of all 5 bakes within a wheat age study (8, 50, 91 days) and treatment group ± SE. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). Mean values followed by the same letter are not significantly different at P = 0.05. Comparisons both within and between storage conditions at a constant wheat age are depicted.

Appendix C - DGDG and MGDG Data

Table C.1 Mean DGDG Levels by Storage Condition over Both Wheat and Flour Age

	8 Day Wheat		50 Day Wheat		91 Day Wheat	
Flour Age (Days)	FZ	RT	FZ	RT	FZ	RT
20	0.19a ± 0.05	0.22a ± 0.05	0.24a ± 0.05	0.20a ± 0.05	0.19acd ± 0.05	0.16ac ± 0.05
23	0.26a ± 0.05	0.27a ± 0.05	0.19a ± 0.05	0.24ad ± 0.05	0.13ac ± 0.05	0.12cd ± 0.05
26	0.19a ± 0.05	0.31a ± 0.05	0.32ab ± 0.05	0.21a ± 0.05	0.40b ± 0.05	0.27ab ± 0.05
29	0.28ab ± 0.05	0.27a ± 0.05	0.19ab ± 0.05	0.26a ± 0.06	0.29bd ± 0.05	0.17ad ± 0.05

Mean DGDG level (signal per mg of defatted flour from bound lipids) ± SE; n=3. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). Mean values followed by the same letter are not significantly different at P = 0.05. Comparisons within flour age, between storage conditions of a constant wheat and flour age, and over wheat age with a constant storage condition and flour age are depicted.

Table C.2 Mean MGDG Levels by Storage Condition over Both Wheat and Flour Age

	8 Day Wheat		50 Day Wheat		91 Day Wheat	
Flour Age (Days)	FZ	RT	FZ	RT	FZ	RT
20	0.29a ± 0.08	0.40a ± 0.08	0.42a ± 0.08	0.34a ± 0.08	0.35ab ± 0.08	0.27a ± 0.08
23	0.26a ± 0.08	0.25a ± 0.08	0.36a ± 0.08	0.35a ± 0.08	0.27ab ± 0.08	0.25a ± 0.08
26	0.28a ± 0.08	0.37a ± 0.08	0.48ab ± 0.08	0.39a ± 0.08	0.57b ± 0.08	0.32a ± 0.08
29	0.27a ± 0.08	0.26a ± 0.08	0.30a ± 0.08	0.31a ± 0.10	0.26a ± 0.08	0.28a ± 0.08

Mean MGDG level (signal per mg of defatted flour from bound lipids) ± SE; n=3. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). Mean values followed by the same letter are not significantly different at P = 0.05. Comparisons within flour age, between storage conditions of a constant wheat and flour age, and over wheat age with a constant storage condition and flour age are depicted.

Table C.3 Mean DGDG:MGDG Ratios by Storage Condition over Wheat and Flour Age

	8 Day Wheat		50 Day Wheat		91 Day Wheat	
Flour Age (Days)	FZ	RT	FZ	RT	FZ	RT
20	0.64a ± 0.20	0.55a ± 0.20	0.58ac ± 0.20	0.58a ± 0.20	0.76ab ± 0.20	0.69ab ± 0.20
23	1.16a ± 0.20	1.06ab ± 0.20	0.52bc ± 0.20	0.68ab ± 0.20	0.53bc ± 0.20	0.5ab ± 0.20
26	0.74a ± 0.20	0.85ab ± 0.20	0.75ab ± 0.20	0.64a ± 0.20	0.85ac ± 0.20	0.84ab ± 0.20
29	1.03ad ± 0.20	1.13bd ± 0.20	0.64ab ± 0.20	0.94ab ± 0.25	1.2a ± 0.20	0.61b ± 0.20

Mean DGDG:MGDG ratio ± SE; n=3. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). Mean values followed by the same letter are not significantly different at P = 0.05. Comparisons within flour age, between storage conditions of constant wheat and flour age, and over wheat age with a constant storage condition and flour age are depicted.

Table C.4 Mean FZ and RT DGDG:MGDG Ratio Levels

Wheat Age (Days)	FZ	RT
8	0.89a ± 0.10	0.90a ± 0.10
50	0.62a ± 0.10	0.71a ± 0.11
91	0.84a ± 0.10	0.66a ± 0.10

Mean DGDG:MGDG ratio of all 4 extractions within a wheat age study (8, 50, 91 days) ± SE. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). DGDG and MGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts. Mean values followed by the same letter are not significantly different at P = 0.05. Comparisons both within and between storage conditions at a constant wheat age are depicted.

Table C.5 Mean FZ and RT DGDG Levels

Wheat Age (Days)	FZ	RT
8	0.23a ± 0.02	0.27a ± 0.02
50	0.23a ± 0.02	0.23ab ± 0.03
91	0.25a ± 0.02	0.18b ± 0.02

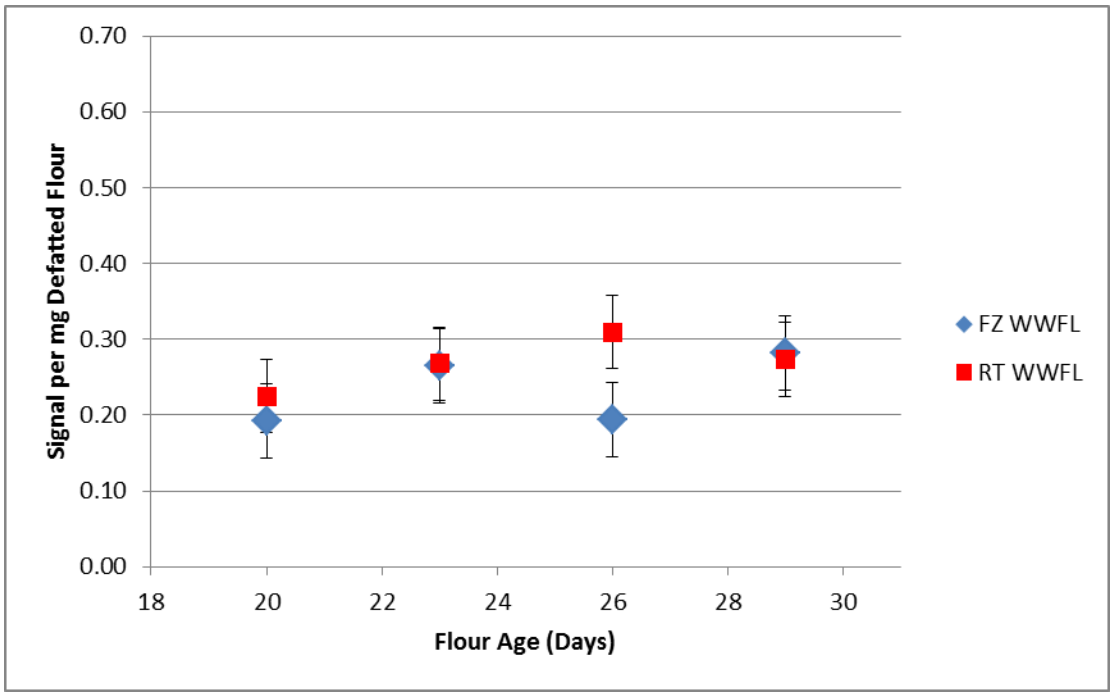
Mean DGDG level (signal per mg of defatted flour extracted from bound lipids) of all 4 extractions within a wheat age study (8, 50, 91 days) ± SE. The wheat and resulting flour was stored at FZ (-26˚C) and RT (23˚C) over time. DGDG levels are the sum of all the different lipid acyl carbon and double-bond configuration amounts. Mean values followed by the same letter are not significantly different at P = 0.05. Comparisons both within and between storage conditions at a constant wheat age are depicted.

Table C.6 Mean FZ and RT MGDG Levels

Wheat Age (Days)	FZ	RT
8	0.27a ± 0.04	0.32a ± 0.04
50	0.39a ± 0.04	0.35a ± 0.04
91	0.36a ± 0.04	0.28a ± 0.04

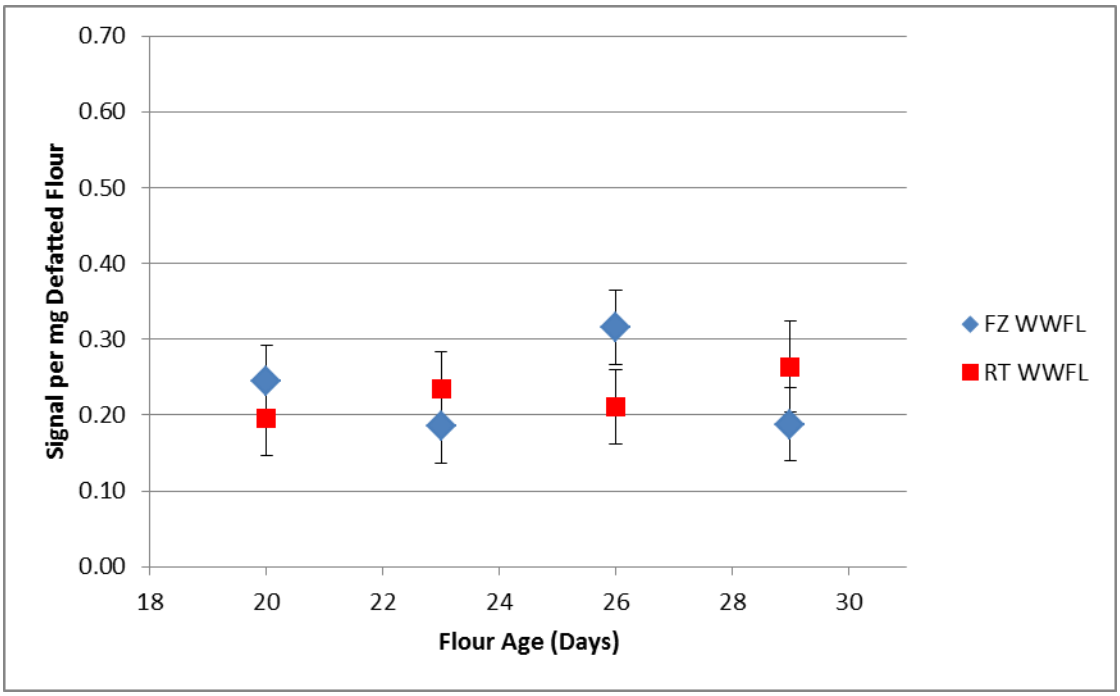
Mean MGDG level (signal per mg of defatted flour extracted from bound lipids) of all 4 extractions within a wheat age study (8, 50, 91 days) ± SE. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). MGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts. Mean values followed by the same letter are not significantly different at P = 0.05. Comparisons both within and between storage conditions at a constant wheat age are depicted.

Figure C.1 Mean 8 Day Wheat DGDG Levels by Storage Condition over Flour Age



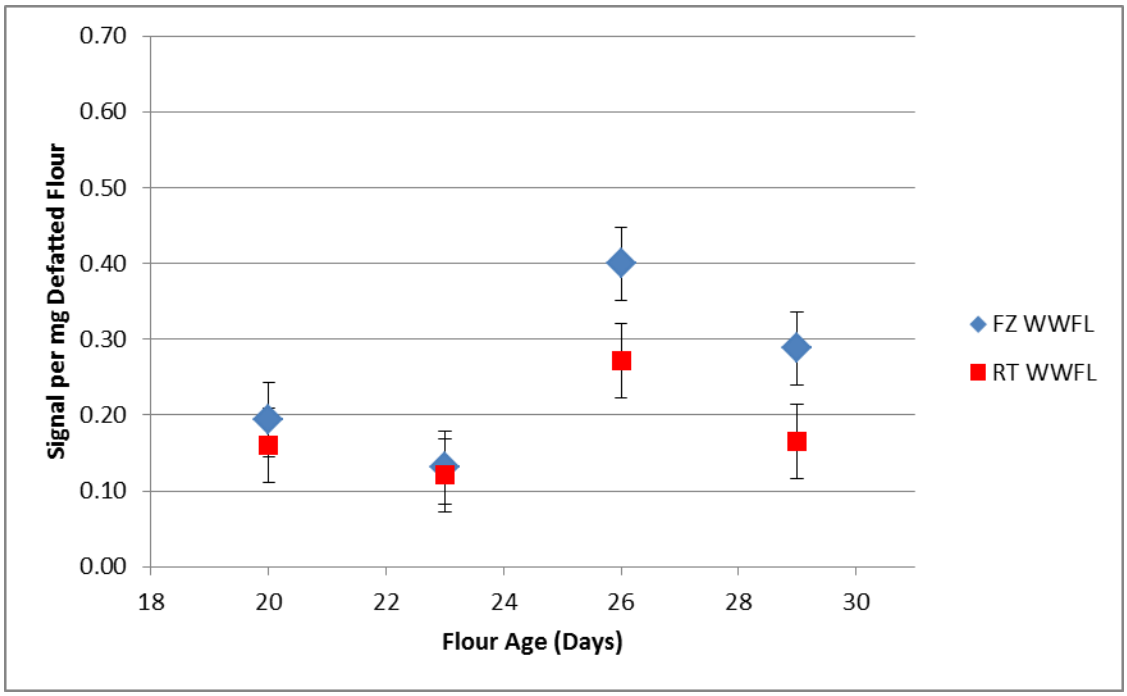
Mean 8 day wheat DGDG level (signal per mg of defatted flour extracted from bound lipids) ± SE; n=3. The whole wheat flour was stored at FZ (-26˚C) and RT (23˚C) over time. DGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts.

Figure C.2 Mean 50 Day Wheat DGDG Levels by Storage Condition over Flour Age



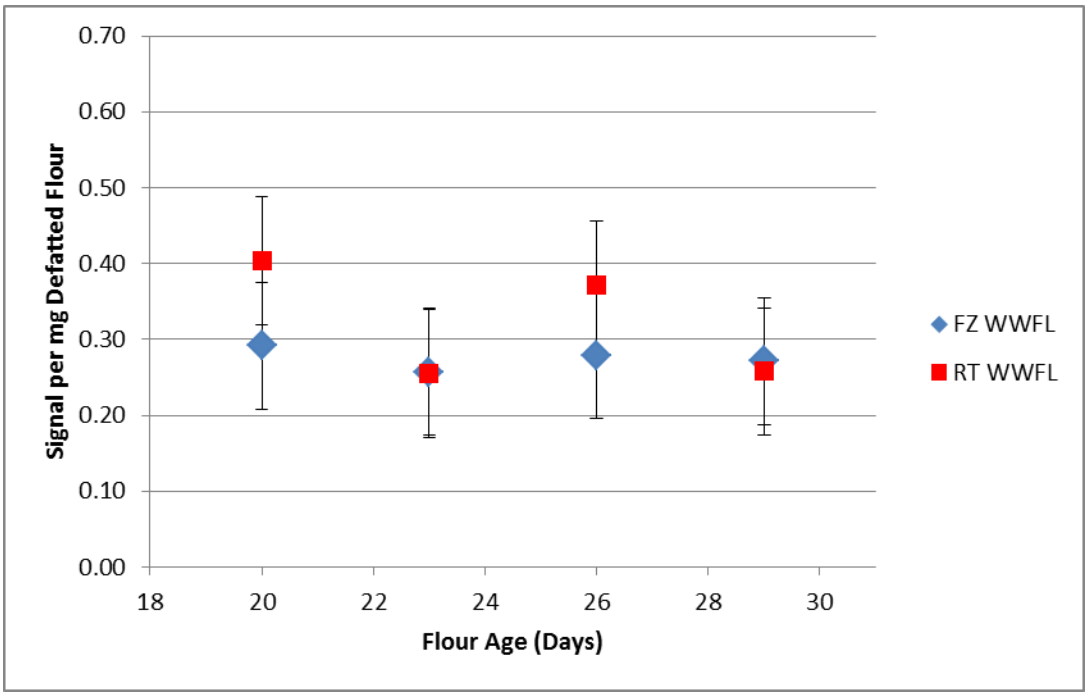
Mean 50 day wheat DGDG level (signal per mg of defatted flour extracted from bound lipids) ± SE; n=3. The whole wheat flour was stored at FZ (-26˚C) and RT (23˚C) over time. DGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts.

Figure C.3 Mean 91 Day Wheat DGDG Levels by Storage Condition over Flour Age



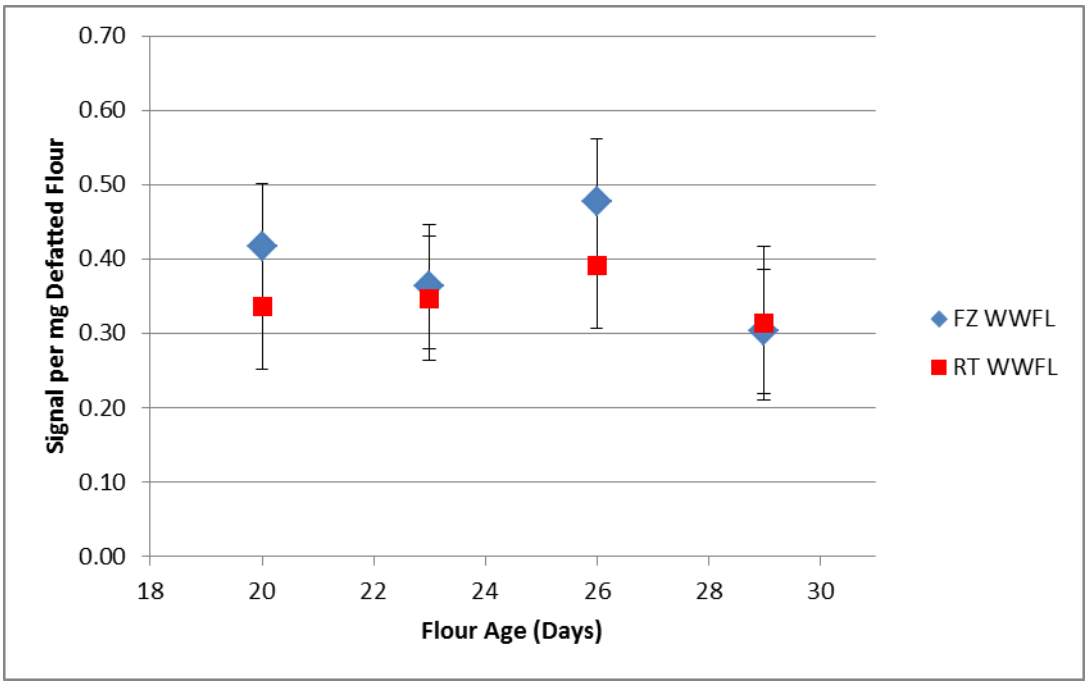
Mean 91 day wheat DGDG level (signal per mg of defatted flour extracted from bound lipids) ± SE; n=3. The whole wheat flour was stored at FZ (-26˚C) and RT (23˚C) over time. DGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts.

Figure C.4 Mean 8 Day Wheat MGDG Levels by Storage Condition over Flour Age



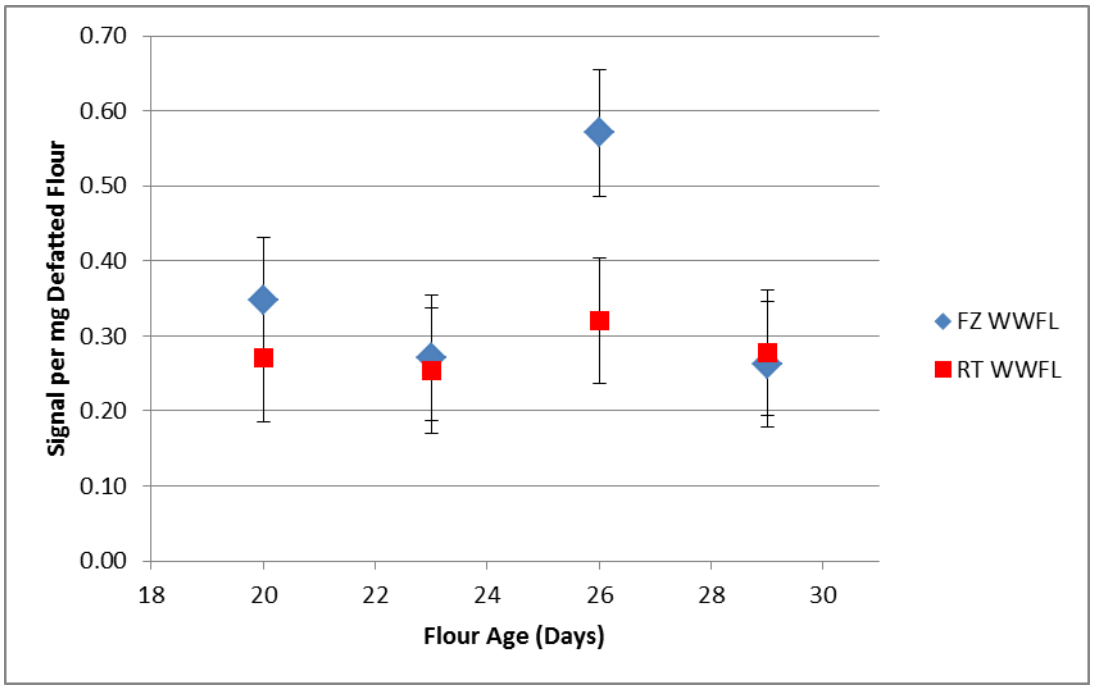
Mean 8 day wheat MGDG level (signal per mg of defatted flour extracted from bound lipids) ± SE; n=3. The whole wheat flour was stored at FZ (-26˚C) and RT (23˚C) over time. MGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts.

Figure C.5 Mean 50 Day Wheat MGDG Levels by Storage Condition over Flour Age



Mean 50 day wheat MGDG level (signal per mg of defatted flour extracted from bound lipids) ± SE; n=3. The whole wheat flour was stored at FZ (-26˚C) and RT (23˚C) over time. MGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts.

Figure C.6 Mean 91 Day Wheat MGDG Levels by Storage Condition over Flour Age



Mean 91 day wheat MGDG level (signal per mg of defatted flour extracted from bound lipids) ± SE; n=3. The whole wheat flour was stored at FZ (-26˚C) and RT (23˚C) over time. MGDG levels were the sum of all the different lipid acyl carbon and double-bond configuration amounts.

Appendix D - 50 Day Wheat Study

One reason for the drop in specific volume at this age was due to larger final loaf weights for the 50 day wheat study compared to the 8 and 91 day studies (Table D.1). For statistical analysis (Table D.2) the RT and FZ 50 day wheat specific volumes were calculated using the average loaf weights from the RT and FZ 91 day wheat studies respectively. When this was done the FZ and RT 50 day wheat specific volumes increased (Table D.2) but FZ 50 day wheat did not show any statistical changes due to the recalculation (i.e., the FZ 50 day wheat specific volumes were still significantly lower than the 8 and 91 day specific volumes (Table D.2)). The RT 50 day wheat did show changes in this case. The specific volumes were statistically equivalent to the RT 91 day wheat study but still significantly lower than the RT 8 day wheat when loaf weights were substituted.

Both the oven and room temperature could have affected the baking results as well. A low baking temperature could have caused the larger loaf weights for the 50 day wheat study resulting in lower specific volumes. The use of a different brand of yeast for the 22 day bake of the 50 day wheat study could have resulted in lower specific volumes.

Table D.1 Change in Specific Volume and Loaf Weight over Wheat Age by Storage Temperature

Wheat Age (Days)	FZ S.Vol (cc/g)	RT S.Vol (cc/g)	FZ Weight (g)	RT Weight (g)
8	4.72a ± 0.02	4.56b ± 0.02	149.61	150.72
50	4.54b ± 0.02	4.44c ± 0.02	152.25	153.01
91	4.72a ± 0.02	4.52b ± 0.02	150.08	151.11

Each whole wheat loaf specific volume (cc/g) represents a mean of all 5 bakes within a wheat age study (8, 50, 91 days) and treatment group ± SE. Storage conditions over both flour and wheat age: FZ (-26˚C) and RT (23˚C). Mean values followed by the same letter are not significantly different at P = 0.05. Comparisons both within and between storage conditions at a constant wheat age are depicted.

Table D.2 Change in Specific Volume after Replacing 50 Day Wheat Loaf Weights

Wheat Age (Days)	FZ S.Vol (cc/g)	RT S.Vol (cc/g)
8	4.72a ± 0.02	4.56b ± 0.02
50	4.60b ± 0.02	4.50c ± 0.02
91	4.72a ± 0.02	4.52bc ± 0.02

Each whole wheat loaf specific volume (cc/g) represents a mean of all 5 bakes within a wheat age study (8, 50, 91 days) and treatment group ± SE. The FZ and RT 50 day wheat study loaf weights were replaced by FZ and RT 91 day wheat weights respectively. Mean values followed by the same letter are not significantly different at P = 0.05. Comparisons both within and between storage conditions at a constant wheat age are depicted.

Appendix E - WWFL Granulation and Feed Rate Data

Table E.1 Mean Feed Rates

	RT WWFL (g/min)			FZ WWFL (g/min)		
Wheat Age (Days)	1st	2nd	3rd	1st	2nd	3rd
8	209.22	1068.21	968.17	266.92	871.30	763.26
50	245.44	1127.31	963.45	207.24	917.01	669.10
91	333.93	1078.96	1118.41	378.23	1015.16	965.35

Mean feed rate in grams/minute for all 3 milling passes for both treatment groups; n=9.5.

Table E.2 Whole Wheat Flour Granulation Data

Cumulative % Held Over						
Opening Size (μm)	91 Day FZ	91 Day RT	50 Day FZ	50 Day RT	8 Day FZ	8 Day RT
0	100	100	100	100	100	100
150	24.67	24.92	25.12	24.5	26.72	24.63
250	9.64	9.88	10.34	9.76	11.14	10.37
420	2.87	3.08	3.59	3.16	4.31	3.77
594	0.9	0.98	1.26	1.01	1.56	1.27
841	0.22	0.25	0.32	0.13	0.49	0.40

The granulation data for 8, 50, and 91 day wheat studies are shown for both RT and FZ storage conditions. Each value is the mean cumulative % held over a given screen analyzed in triplicate. All the WWFL was combined and blended before particle size analysis for each storage condition within an aging period.

Appendix F - Mixing Times and Water Absorptions

Table F.1 Mixing Times and Water Absorptions Used for Final Experimental Bakes

	FZ		RT	
Wheat Age (days)	Mixing Time (min)	Absorption (%)	Mixing Time (min)	Absorption (%)
8	7.50	79	7.0	79
50	7.50	79	7.0	79
91	7.25	79	7.0	79

Optimized baking process for each wheat age study.

The water absorption did not change over wheat age for WWFL stored at FZ and RT. The mixing time of flour stored at RT stayed constant at 7.0 minutes of mixing. The mixing time of flour stored at FZ was 7.5 minutes for the 8 and 50 day wheat but decreased to 7.25 minutes for the 91 day wheat study.