EFFECTS OF POSTMILLING TIME AND TEMPERATURE ON THE BREADMAKING QUALITY AND LIPIDS OF WHOLE WHEAT FLOUR

by

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Abstract

This work investigated the relationship between flour age (days post-milling), storage condition (temperature), and the bread baking quality of whole wheat flour. A laboratory scale milling method was designed to mimic the particle size distribution of commercially milled whole wheat flours and the 100 g 'pup' loaf baking method was adapted for use with whole wheat doughs. Laboratory milled whole wheat flour (Karl 92) was subjected to a 21 day storage study at two storage temperatures (72 & -15 F) with quality (baking) and chemical (lipids) analyses conducted every three days. Parameters for quality analysis included: loaf weight, volume & specific volume, as well as slice area, cell number, wall thickness, cell diameter, elongation, and non-uniformity. Three lipid classes (glycolipids, phospholipids, and neutral lipids) were extracted and analyzed by TLC with quantification by computerized analysis of spot size and density. Results were analyzed by ANOVA.

Analysis of the loaf quality data revealed no trends in volume or specific volume as a function of storage time or temperature, although values for some specific days were significantly different. Likewise, analysis of crumb characteristics revealed no consistent trends for either time or storage temperature. Again, values for some, but not all, parameters (area, brightness, wall thickness, cell diameter, and non-uniformity) were significantly different for specific days of the study. Analysis of lipids revealed no consistent trends for either time or storage temperature. However, values for some lipid classes (total glycolipids, free phospholipids, and total phospholipids) were significantly different for storage temperature, and values for total neutral lipids were significantly different for specific days of the study.

Suggested future research opportunities include: using new crop wheat, increasing storage duration, performing WW flour lipid exchange studies, and using lipid profiling to identify and more closely track changes in individual lipid species.

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Dedication

To God, the Bread of Life, who taught me how to roll with the punches.

CHAPTER 1 - Introduction and Literature Review

Introduction

The USDA issued a Dietary Guidelines recommendation on June 12th, 2005 for consumers to increase whole grain consumption. However, it is generally recognized that whole wheat flours containing the germ can develop problems due to lipid changes. White flour, especially soft wheat flour destined for use in cakes, increases in quality with time after harvest and milling, as indicated by a desirable drop in batter specific gravity and increase in batter viscosity, distilled water binding capacity (DWBC), minimum batter viscosity during heating, and cake volume. Quality increases with post-milling aging of flour up to a certain duration of storage, after which these parameters reach a plateau (Shelke et al., 1992a; Shelke et al., 1992b). However, in the baking industry, whole wheat flour is thought to decline in quality with storage time after milling (Jon Faubion, personal communication). The goals of this research were to rigorously test for and document any post-milling time-related changes in whole wheat flour baking quality and flour lipids, mainly lipid oxidation and breakdown, as monitored by Thin Layer Chromatography (TLC). Lipid changes are expected to accompany deleterious changes in bread baking quality (such as volume, crumb grain, and dough handling properties). A second objective was to test for relationships between any changes in bread baking quality and changes in the glycolipid, phospholipid, and neutral lipid fractions of the flour. The research conducted here should prove useful to the food industry by providing information for controlling quality and processing variability in whole wheat flour based products. The hypothesis is that changes occur with time in whole wheat and that they are manifested in the lipid fraction.

Literature Review

Classifications of Flour Lipids

Lipids Overview

Lipids are classified mainly based on their solubilities rather than their chemical structure (Gurr et al., 2002). Fats, shortening, vegetable oils, and lipids are all names for glycerol esters of fatty acids (Sluimer, 2005). Fats are solid at room temperature, while oils are liquid at room temperature. A lipid is "a chemically heterogenous group of substances having in common the property of insolubility in water, but solubility in non-aqueous solvents such as chloroform, hydrocarbons, or alcohols" (Gurr et al., 2002). Wheat flour contains a variety of lipid types, generally totaling 2-3% of refined (white) flour (Glass, 1960), or 2-4 % of the entire wheat kernel (Morrison, 1976, 1978a, 1978b). The exact percentages and compositions of wheat flour lipids vary depending on the extracting solvents, wheat variety, growth conditions, wheat age pre- and post-harvest, and milling method (Glass, 1960). The typical composition of a hard wheat kernel is: 14 % moisture, 12 % protein, 60 % starch, 10 % fiber, 2% lipids, and 2% minerals (Sluimer, 2005). The lipid distribution within the kernel is: 1-2% in endosperm, 8-15 % in germ, and ~6 % in bran (Pyler, 1988; Morrison, 1976, 1978a, 1978b). White flour contains less than 1 % lipids, while WW flour contains 2% lipids (Sluimer, 2005). Lipids are present mainly in the germ fraction, which is 12 % lipid (Sluimer, 2005). Germ oil contains 15.5 % solid FA's, 25.5 % oleic acid, 52.6 % linoleic acid, and 6.4 % linolenic acid as mixed glycerides (Pyler, 1988). Wheat germ lipids are 80 % nonpolar triglycerides, monoglycerides, diglycerides, and free fatty acids (TG's, MG's, DG's, FFA's) and 19 % saturated (Pyler, 1988). Lipid nomenclature changes over the years and the advent of new nomenclature must be kept in mind in reviewing the literature (Gurr et al., 2002). Thus, lecithin is now termed "phosphatidylcholine," and triglycerides are now termed "triacylglycerols" (Gurr et al., 2002).

Starch/ Non-Starch

Lipids can also be classified by their location, as either starch or non-starch bound lipids (Morrison, 1978a; MacRitchie et al., 1973). Non-starch lipids include steryl esters, TG's, DG's, MG's, FFA's, esterified steryl glucoside, MGDG, DGDG, and all the types of phosphoglycerides

(Morrison, 1978b). Non-starch lipids require room temperature extraction with polar solvents, such as acetone (glycolipids and traces of acidic phospholipids) and methanol (phospholipids) (Gurr et al., 2002). Non-starch lipids participate in all physical, chemical, and biochemical reactions concerning flour lipids and dough lipids (Morrison, 1978b). Lipids are quite stable while still in the intact kernel. In damp stored flour, non-starch lipids undergo hydrolysis either enzymatically or through autoxidation, to form FFA's.

Starch lipids include nonpolar classes, FFA's, glycolipids, lysophospholipids, and lysophosphatidylcholine (Morrison, 1978b). Starch lipids are extracted with hot butanol-water mixtures (Morrison, 1978b). Starch lipids are virtually inaccessible, i.e. inert, when in flour; and are stable against autoxidation even when stored in an aerobic environment and at high temperature (Morrison, 1978b).

Free/ Bound

Non-starch lipids are further classified by their extractability, as free lipids or bound lipids. Free lipids comprise 60% of flour lipids, while bound lipids comprise 40% of flour lipids (Hoseney et al., 1969).

Free lipids can be extracted with a nonpolar solvent (Pyler, 1988), such as light petroleum ether, diethyl ether (Morrison, 1978b), water saturated *n*-butanol, benzene-ethanol-water mixture, or ethanol-diethyl ether-water mixture. They are mostly TG's and FFA's, consisting of ~60% neutral lipids (non-polar), ~25% polar glycolipids, ~15% phospholipids, and also carotenoids. Free lipids compose 0.8% of patent wheat flour; of that, 0.6% are nonpolar (TG's) and 0.2% are polar (2/3 glycolipids, 1/3 phospholipids) (Pyler, 1988; Pomeranz, 1988). Free lipids make up 90% of the nonpolar lipids, and 20% of the polar lipids (Pyler, 1988). Free lipids consist of 25% polar lipids and 75% nonpolar lipids.

Bound lipids are the non-starch lipids remaining after extraction of free lipids (Morrison, 1978b). Lipids can be bound to protein or starch. Bound lipids are not extracted with ether. Instead, they require a cold polar solvent (Pyler, 1988), such as water-saturated butanol (WSB). Bound lipids make up ~1.0% of patent wheat flour weight, of which 2/3 is polar (phospholipids and glycolipids) (Pyler, 1988; Pomeranz, 1988). They also contain monoacyl lipids. Bound lipids are also present in the gluten fraction of flour (Hoseney et al., 1969).

Special Binding

Starch lipids are stable against autoxidation, and are extracted with hot butanol-water mixtures (Glass, 1960). However, solvents may alter the functional properties of flour proteins (Glass, 1960). Hydration (i.e. dough mixing) causes all of the free polar lipids and half of nonpolar lipids to become bound to gluten, resulting in lipoprotein complexes (mainly w/ glutenin) (Pyler, 1988). To extract flour lipids present as lipoproteins requires solvent treatment with water-saturated 1-butanol, aqueous ethanol, or acid, followed by dissolving the separated lipid in a solvent such as chloroform or petroleum ether (Glass, 1960). Lipoproteins can be isolated from wheat flour lipids using petroleum ether and salt solutions (Hoseney et al., 1970b). Lipoproteins apparently have no effect on breadmaking. The primary effects on breadmaking are due to the polar lipids (Hoseney et al., 1970b). Flour hydration, caused by excess atmospheric moisture during storage, mixing, or gluten washing, also causes some nonpolar free lipids to bind to starch, thus changing their extractability (Glass, 1960; Pomeranz, 1988).

Lipid interactions occur and change during mixing and baking. Lipid-protein bonds formed during mixing weaken during baking, and lipids can then translocate to starch (amylose and amylopectin) (Pyler, 1988). Lipid-starch interactions can then occur. Saturated monoacyl lipids form amylose-inclusion complexes (Morrison, 1978b). Linoleic acid is known to interact with the starch fraction of flour (Pyler, 1988). Free lipids interact with proteins during mixing and with non-protein components after the mixing stage of breadmaking. Bound lipids can bind to gluten proteins during dough mixing. Polar lipids interact with carbohydrates (CHO's) and proteins through hydrophilic bonds (Pyler, 1988). Non-polar lipids interact with other dough constituents through hydrophobic bonds and Van der Waal's forces (Pyler, 1988). The majority of lipids have become bound by the completion of the dough mixing process.

Polarity

Flour lipids are also grouped by their polarity, as polar lipids, intermediate polarity lipids, or nonpolar lipids (MacRitchie et al., 1973; Chung et al., 1978). Polar lipids are amphipathic. They contain a polar head and a hydrocarbon tail (Pyler, 1988). Of the total wheat flour lipids, ~51% are nonpolar and ~49% are polar (glycolipids 26.4%, phospholipids 22.7%) (Pyler, 1988).

Polar lipids comprise 0.2 % of patent wheat flour and are primarily glycolipids and phospholipids (Pomeranz, 1988). There is a higher protein and polar lipid (i.e. phospholipid)

content in fine granule starch due to the higher surface area of the smaller starch particles. Polar lipids are present in the acid-soluble fractions of flour. Polar lipids are eluted with polar solvents such as methanol (Pomeranz, 1988). Intermediate polarity lipids include monoglycerides, free fatty acids, monogalactosyl diglyceride, and digalactosyl diglyceride.

Nonpolar lipids are known to be deleterious to loaf volume, but are minimally present in flour, composing 0.6 % of patent wheat flour (Pomeranz, 1988). They are extracted from flour using chloroform (Pomeranz, 1988; Gurr et al., 2002). The nonpolar fraction includes mainly triglycerides, but also contains hydrocarbons, sterols, steryl esters, diglycerides, and free fatty acids. They are present in acetic acid-soluble fractions of flour (Chung et al., 1978; De Stefanis et al., 1976; MacRitchie et al., 1973; Pomeranz, 1988).

Phospholipids

Phospholipids, or phosphoglycerides, are composed of a glycerol backbone esterified to phosphoric acid and two FA's (Pyler, 1988). Phospholipids in flour are (from high to low content) lysophosphatidyl cholines, phosphatidyl cholines, *N*-Acyl phosphatidyl ethanolamines, and *N*-Acyl lysophosphatidyl. ethanolamines, lysophosphatidyl ethanolamines, phosphatidyl ethanolamines, phosphatidyl serines, phosphatidyl inositol (Pyler, 1988). Of the phospholipids, phosphatidylcholine is the most abundant phospholipid in wheat, comprising 45.5% of the phospholipids in the whole kernel wheat (Morrison, 1978b). Starch lipids are composed primarily of lysophospholipids. They are highly resistant to autoxidation (Pyler, 1988).

Glycolipids

Glycolipids consist of one or more sugars with a glucosidic linkage to an unesterified hydroxyl group of DGs (Pyler, 1988). Glycolipids are also called glycolsylglycerides, and are the predominant lipids of photosynthetic membranes of higher plants, algae, and cyanobacteria (Gurr et al., 2002). Because these membranes are so prevalent, MGDG is the most abundant lipid in the world, followed by DGDG (Gurr et al., 2002). Flour glycolipids (in order of highest to lowest content) are DGDG's, MGDG's, *o*-Acylmonogalactosyl DG's, steryl glucosides and ceramide DG's, 6-*o*-Acylsteryl glucosides, DGMG's, MGMG's, and ceramide diglucosides (Pyler, 1988). Wheat glycosyl glycerides contain MGDG, DGDG, esterified (6-*o*-acyl) monogalactosyl diglyceride (EMGDG), and small amounts of MGMG and TGDG (Morrison, 1978b). MGDG is comprised of 2-position FA's which are highly unsaturated and contain 83%

linoleic acid and 7% linolenic acid (Morrison, 1978b). EMGDG is esterified to saturated FA's (Morrison, 1978b). MGDG contains a high proportion of PUFA's; however the types of PUFA's and percent composition depend on species of plant and growing conditions (Gurr et al., 2002). DGDG also contains a high proportion of PUFA's, especially 18:3 (Gurr et al., 2002). Glycolipids "combine the polar features of polyols with the lipophilic behavior of extended aliphatic chains" (Law, 1960). They can be soluble in both hydrophobic and aqueous solvents, although WSB is believed to be a superior solvent to ethanol-ether mixtures (Law, 1960). Glycolipids may be part of the structure of lipid-water interfaces, and may form high molecular weight polymers or micelles, as examined by ultracentrifugation (Law, 1960). Galactosyl glycerides exist in wheat gluten. Bleaching may degrade digalactosyl glycerides to monogalactosyl glycerides (Law, 1960).

Free polar lipids, mainly glycolipids, are bound to glutenin hydrophobically and to gliadin hydrophilically (Hoseney et al., 1970a; Wehrli et al., 1970). These bonds aid in the structure of gas-retaining complexes in gluten (Hoseney et al., 1970a). If gluten is unfractionated, then glycolipids bind to both gliadin and glutenin at the same time (Hoseney et al., 1970a). Gluten in dough is unable to retain CO_2 if free lipids are removed but is able to retain CO_2 once again when the polar fraction is returned to the flour (Hoseney et al., 1970a).
The galactosyl moiety of GL's are bound by hydrogen bonds to the polar amino acid residues of gliadin (Wehrli et al., 1970). In dough, this bond consists of hydrogen bonds (Wehrli et al., 1970). However, in the presence of water, the GL's are bound to the gliadin by hydrophobic bonds (Wehrli et al., 1970). Infrared spectroscopy reveals that hydrogen bonds exist between glycolipids, gelatinized starch, and gluten components (Wehrli et al., 1970). Van der Waals bonds exist between glycolipids and gluten components (Wehrli et al., 1970). NMR spectra show hydrogen bonds with starch, and hydrophobic bonds (and some H-bonds) with gluten (Wehrli et al., 1970). Hydrogen bonds are sensitive to heat (Hoseney et al., 1970a). Heat therefore affects the hydrogen bonds of GL's to gliadin. This is important because gliadin controls loaf volume (Hoseney et al., 1970a). However, hydrophobic bonds are very stabile in the presence of heat. This is important because glutenin is hydrophobically bound to GL's (Hoseney et al., 1970a).

Neutral Lipids

Flour nonpolar lipids (in order of highest to lowest content) contain TG's, steryl esters, FFA's, 1,2-diglycerides, 1,3-diglycerides, free sterols, and monoglycerides (Pyler, 1988). About 70% of the total fatty acid in wheat is polyunsaturated linoleic acid (Sluimer, 2005). TG's are the main lipid fraction in all wheat fractions except the starch fraction (Morrison, 1978b).

Role of Flour Lipids in Bread Baking Quality

Dough mixing binds all of the free polar lipids and 50% of the free nonpolar lipids (Hoseney et al., 1969). Removing free flour lipids, while adding shortening to the formula, causes reduced loaf volume and crumb softness (Pyler, 1988). The addition of free lipids at low levels causes increased bread firming and increased loaf volume, while the addition of free lipids at high levels causes delayed bread firming and decreased loaf volume (Rogers et al., 1988).

Extraction of bound lipids damages both dough and bread quality (Hoseney et al., 1969). The presence of bound lipids is necessary for the gluten proteins to retain CO_2 during fermentation (Pyler, 1988). However, increased added lipid content in a dough requires the addition of more oxidative flour improvers (Sluimer, 2005).

Polar Lipids

Polar lipids are beneficial to baking (Pyler, 1988). They contribute positively to dough handling and bread loaf volume and texture (Graybosch et al., 1993). The removal of native polar lipids from flour causes shortening to have deleterious effects on bread loaf volume and crumb grain (Pyler, 1988). However, the combination of polar lipids and shortening has beneficial effects on bread quality (Pyler, 1988).

According to Hoseney et al (1969), free polar lipids increase volume more effectively than do bound polar lipids. Of the free polar lipids, galactosylglycerides were the most effective at restoring loaf volume. Bound polar lipids caused a slight increase in loaf volume, and performed differently in their native state than when extracted and reconstituted. Free and bound nonpolar lipids caused decreased loaf volume and impaired crumb grain; which was restored by combining polar lipids with them.

Phospholipids

Lecithin, or phosphatidylcholine, (from soy) has been shown to protect against decreases in loaf volume due to addition of ingredients such as soy protein. Of the phospholipids in soy, phosphatidylcholine was the most effective. According to Ukai and Urade (2007), phosphatidylcholine "increases the gas-retaining ability of dough, the dough volume on fermentation and the loaf volume of bread." The same study also noted that phophatidylcholine acts synergistically with glycolipids to stabilize the foam and increase the gas-retaining ability of the dough (Ukai et al, 2007). Phosphatidylcholine employs its bilayer structure in the liquid-crystalline state to function in bread dough and can be used to increase loaf volume in doughs containing soy protein isolate (Urade et al., 2003). Certain combinations of phospholipids have been shown to work synergistically to increase loaf volume even more than individual phospholipids (Helmerich et al., 2005).

Glycolipids

Polar lipids function to improve loaf volume and crumb grain, and to maintain bread freshness (Pomeranz et al., 1965; Pomeranz et al., 1966). These positive bread qualities have been attributed specifically to the glycolipid portion of the polar lipids (Daftary et al., 1968; Ponte et al., 1969). Glycolipids include MGDG and DGDG; DGDG is the component mainly responsible for increased bread quality (Pyler, 1988). Because glycolipids are a type of nonionic surfactant, studies were conducted to test the ability of other surfactants to replace shortening in bread and to counteract negative effects of soy flour (Hoseney et al., 1972). The results indicated that replacement of total free flour lipids with emulsifiers could: increase loaf volume (sucrose monotallowate and/or sucrose monopalmitate), maintain loaf volume (sodium or calcium stearoyl-2-lactylates), or maintain loaf volume but cause adverse effects on crumb grain (pluronic polyols F-108 and F-68). Dose-dependent correlations of baking functionality and chemical structure of separate glycolipids were identified (Pomeranz et al., 1969). The optimum hydrophilic-lipophilic balance (HLB) for glycolipids relative to improving baking performance was determined to be 12.2 for octanoic acid as the optimum fatty acid chain length for synthetic MGDG (monogalactosyl dilinoleylglycerol) (Selmair et al., 2008). This value is close to 12.0, the optimum emulsion stability HLB number for o/w emulsions (Selmair et al., 2008). The optimum HLB was determined to be 11.2-11.4, which was best achieved with palmitic acid, but

closely followed by the experimenters' synthetic MGMG (monogalactosyl monolinoleylglycerol), with an HLB value of 10.3-10.9 (Selmair et al., 2008). In that study, of the synthetic glycolipids tested, monogalactosyl monolinoleylglycerol had the best baking activity and antistaling effect, and an HLB range of 8 to 12 for surfactants resulted in the best baking performance (Selmair et al., 2008). However, digalactosyl monolinoleylglycerol also had an acceptable performance, similar to that of commercial surfactants (Selmair et al., 2008).

During dough mixing, glycolipids form a complex. Gliadin hydrophilically bonds to glycolipid, which in turn hydrophobically bonds to glutenin (Pyler, 1988). This complex acts to retain gas bubbles in dough (Pyler, 1988). However, the complex is affected by baking. Its hydrogen bonds are heat labile, but its hydrophobic bonds are strengthened with heat (Pyler, 1988). Glycolipids may also bind with starch to retain gas, forming a starch-glycolipid complex, which aids in bread loaf freshness retention (Pyler, 1988). The butanol in water-saturated butanol (WSB) complexes with starch, halts gas production, and denatures the gliadin fraction (Hoseney et al., 1969).

Neutral Lipids

Nonpolar lipids are have detrimental effects on gluten quality and are known to be deleterious to baking by causing decrease in loaf volume (Chung et al., 2002; Pomeranz, 1988). The effect on volume may be masked by the addition of 3% lard because lard contains saturated fats, while deleterious effects to volume are caused by unsaturated fats (De Stefanis et al., 1976). Shortening, even though it does not complex with amylose, reduces the firming rate of bread by interacting with the native flour lipids (Rogers et al., 1988). Shortening or surfactants aid in the expansion of dough above a temperature of 55 °C and in the retention of carbon dioxide in dough (Moore et al., 1986).

The following FA's account for 97% of the total wheat FA's: linoleic, palmitic, oleic, and linolenic (Pyler, 1988). Lipid function is specific to the fatty acid (Sluimer, 2005). Of the non-polar lipids, FFA's are known to be the most detrimental to loaf volume (Pyler, 1988). Of the free fatty acids, linoleic acid has a negative effect on bread volume, apparently by affecting both starch and gluten (Pyler, 1988). Linoleic acid takes up O_2 through its double bonds, which aids in the oxidation of protein thiol groups, which then aids in gluten development (Sluimer, 2005). Although detrimental effects on bread loaf volume are primarily caused by unsaturated fatty

acids, palmitic acid is also known to cause a slight decrease in loaf volume in the absence of lard (De Stefanis et al., 1976). Monoglycerides are known to complex with starch to decrease the firming rate of bread through a mechanism separate from the way that shortening decreased firming rate (Rogers et al., 1988).

Both saturated and unsaturated fatty acids can contribute to decreased loaf volume at levels as low as 0.5% (FWB), but only unsaturated fatty acids decrease internal crumb score. Palmitic acid is the only saturated FA present in a considerable amount (Pyler, 1988). Unsaturated fatty acids have a more negative effect on loaf volume than do saturated fatty acids. These detrimental effects could be due to the physical state of the fatty acids, which are above their melting point. Increased unsaturation of FA's causes a decreased melting temperature range (Sluimer, 2005). Lipolysis products of unsaturated fatty acids, saturated fatty acids, and shortenings all have a negative effect on consumer acceptability (Miller et al., 1948). Wheat germ contains at least 10% oil, of which 84% is unsaturated fatty acid (Zhou et al., 2007). Oxidation of unsaturated FA's results in rancidity (Sluimer, 2005). Wheat germ, when stored alone, can oxidize and rancidify in a matter of days, due to lipoxidase activity (Zhou et al., 2007). When *cis*-unsaturated FA's are added to fresh flour in the Chorleywood Bread Process (CBP), the bread loaf volume decreases proportionately to the concentration (Warwick et al., 1979).

Fats added to bread dough have several functions, which are not possessed by oils (Sluimer, 2005). Saturated FA's have many positive functions in baking, including better machinability, more flexible dough, more stability at the end of the proofing stage than without added fat, increased oven spring which results in increased specific volume, finer and softer crumb, decreased crumbliness, and slower firming rate (Sluimer, 2005). If the melting range of a saturated FA used for baking is too high, it cannot be properly dispersed in the dough to give the desired positive effects (Sluimer, 2005). The ideal melting range is ~30 °C, and the added fat must be a higher temperature than dough (Sluimer, 2005). Greater effects from the addition of saturated FA's are seen in a short fermentation vs. a long fermentation, and added fats are seen as very necessary, especially in the short fermentation (Sluimer, 2005). At least 1 % added fat (baker's %) is needed for the minimum positive effects on dough (Sluimer, 2005).

Watanabe et al (2003) found that oil (in their study: corn oil) binds to the gluten, aggregates gluten, causes elastic behavior, and causes decreased loaf volume. Fat (shortening)

distributes evenly between starch granules in dough, causes decreased friction between the starch granules, causes more viscous behavior, causes increased loaf volume, and facilitates a thin gluten gel layer (Watanabe et al., 2003). Therefore, Watanable et al (2003) concluded that "thin expandable gluten films and the uniform dispersion of gluten and starch granules in the dough are prerequisites for attaining better baking performance."

Emulsifiers

According to Gomez et al (2004), positive effects of emulsifiers on bread volume are seen only with long proofing times. To delay firming during bread storage, the best emulsifiers are monoglyceride, and lecithin supplemented in lysophospholipids (Gomez et al., 2004). Other emulsifiers include DATEM, SSL, and polysorbate (Gomez et al., 2004). Emulsifiers act as dough strengtheners. They strengthen the dough by forming a complex with gluten proteins (Gomez et al., 2004). Emulsifiers act during the proofing stage and are, therefore, best to use only with long proof methods (Gomez et al., 2004). Emulsifiers cause increased volume and better crumb structure and texture (Gomez et al., 2004).

Oxidative Breakdown of Fatty Acids

When flour is stored under unfavorable conditions such as high moisture and high temperature (Miller et al., 1948), or with exposure to light, enzymes, metals, metalloproteins, or microorganisms (St. Angelo, 1996), oxidative changes can happen, resulting in oxidation products, that can cause negative effects such as decreased dough extensibility and poor bread volume, flavor, and texture (Miller et al., 1948), off-odors and off-flavors (St. Angelo, 1996), and denaturation of protein due to the free radical products of oxidation (Warwick et al., 1979).

Enzymatic or nonenzymatic oxidation of unsaturated fatty acids leads to primary and secondary products that can adversely affect lipid functions in foods (St. Angelo, 1996). This process is also referred to as "autoxidation" (not if enzyme mediated) if the system contains oxygen (St. Angelo, 1996). Lipid autoxidation occurs through a free-radical initiated reaction sequence including initiation, chain propagation, and termination (St. Angelo, 1996). Unsaturated fatty acids, "when oxidized, can form an autocatalytic process, that is, the oxidative products so formed can further catalyze the reaction, which causes the rate to increase with time" (St. Angelo, 1996). Any of the following may initiate autoxidation: temperature, singlet oxygen, photosensitizers, the physiological reduction of oxygen to superoxide radical, or radiation (St.

Angelo, 1996). The degree of fatty acid unsaturation affects susceptibility to oxidation because they contain allylic sites with double bonds that allow "stabilization of free radicals through delocalization of the unpaired electron" (St. Angelo, 1996). The initial reaction produces fatty acid hydroperoxides, which then form secondary products, including aldehydes, ketones, alcohols, acids, or hydrocarbons. These products are encompassed in the term "oxidative rancidity" (St. Angelo, 1996). Results of lipid oxidation in food include unpleasant flavors, odors, aromas, and/or tastes (St. Angelo, 1996).

Enzymes are biological catalysts, "a material that increases the rate of a reaction without being consumed in the reaction or undergoing any permanent changes" (Sluimer, 2005). There are many enzymes that are present in flour or are added to a bread recipe (Sluimer, 2005). Sources of enzymes include malt flour, enzyme-active soy flour, commercial enzyme preparations, and yeast (Sluimer, 2005). The main classes of enzymes in bread doughs are amylases, hemicellulases, proteases, lipases, lipoxygenases, and oxidases (Sluimer, 2005). Lipolytic enzymes act on all accessible simple glycerides, galactosyl glycerides, and phosphoglycerides, to produce FFA's (Morrison, 1978b). These enzymes, include native flour acyl-hydrolases as well as enzymes from molds present in the flour (Morrison, 1978b).

Of the lipid degrading enzymes present in wheat flour, the most important oxidizing enzymes are lipase and lipoxygenase. Lipase and lipoxygenase are natural components of good quality wheat flour (Pyler, 1988). Although inactivated during baking (Miller et al., 1948), lipase and lipoxygenase have synergistic effects on oxygen-uptake and free fatty acid increases during flour storage. In stored wholemeal, lipase in bran acts at low water activity to hydrolyze triglycerides to produce polyunsaturated free fatty acids, which are then oxidized by lipoxygenase at low water activity (Galliard, 1986).

Lipase hydrolyzes flour lipids to produce FFA's (Pyler, 1988). Lipases are specific as to which fats they hydrolyze and at which position(s) in the structure. Lipase hydrolyzes the FA ester bonds at positions 1 and 3 of a TG (Gurr et al., 2002). TG lipases cannot hydrolyze the ester bond at the 2 position, so they produce 2-monoacylglycerols (Gurr et al., 2002). Some lipases hydrolyze not only TG's but also the 1-position of phosphoglycerides (Gurr et al., 2002). Lipase can come from many sources: malt extract, yeast, wheat germ, and soybean flour (Miller et al., 1948). Lipase is present mainly in the aleurone layer, with a smaller presence in the endosperm (Pyler, 1988). Lipase is not frequently added to bread dough (Sluimer, 2005).

Lipase, however, improves baking quality because it hydrolyzes TG's to produce MG's and DG's, which act as emulsifiers (Sluimer, 2005). Lipase does not only act on lipids. There is a specific group of lipases, called glycosidases, which act on glycolipids. Exoglycosidases of known specificity cleave the residues one at a time from non-reducing ends to produce a sequence of monosaccharides and to reveal the position and configuration of glycosidic bonds (Nelson et al., 2000).

Lipoxygenase (also known as lipoxidase) is present in both plants and animals (Gurr et al., 2002). Plant sources include peas, beans, cereal grains, and oil seeds (Gurr et al., 2002). Lipoxygenase exists in two types (Sluimer, 2005). Type 1 oxidizes conjugated double bonds in the PUFA's of flour lipids, while Type 2 produces a bleaching effect as it oxidizes carotenoids & chlorophylls (Sluimer, 2005). Lipoxygenase peroxidizes polyunsaturated fatty acids (PUFA's) such as linoleic and linolenic acid and their monoglycerides to produce FFA's and hydroperoxides w/ polar groups (Pyler, 1988; Miller et al., 1948). The polar groups allow hydroperoxides to interact with carotenoids, tocopherols, and proteins, resulting in bleached carotenoids by the hydroperoxides' oxidation of sulfhydryl groups (which causes flour whitening), destruction of vitamins, and oxididation of protein thiol groups (which result in better dough properties) (Pyler, 1988; Miller et al., 1948). Lipoxygenase can be added to doughs as a component of enzyme-active soy flour. The addition of 1% (baker's %) of enzyme-active soy flour is sufficient to cause bleaching (Sluimer, 2005). The hydroperoxides further decompose to generate secondary oxidation products (Warwick et al., 1980). The breakdown of hydroperoxides into aldehydes, ketones, and alcohols results in oxidative rancidity (Pyler, 1988).

Lipid peroxidation, like other radical chain reactions, proceeds via: initiation, propagation, and termination (Gurr et al., 2002). In the first step, a hydrogen is abstracted from a *cis, cis*-methylene interrupted diene , forming a peroxide radical (Gurr et al., 2002). In the second step, the resulting free radical, a *cis, cis*- methylene interrupted, is then rearranged to form a free radical called a *trans, cis*-conjugated diene (Gurr et al., 2002). In the third and final step, the free radical is then peroxidized, resulting in a *trans, cis*-conjugated diene hydroperoxide (Gurr et al., 2002).

During flour storage, lipoxygenase acts on free lipids. During dough mixing, it acts on free or bound 18:2 and 18:3 lipids, mainly palmitic and linoleic acid. Lipoxygenase increases the mixing tolerance of dough by creating free radicals on linoleic acid, which offsets the action

of oxidants (Pyler, 1988). During dough mixing, lipoxygenase oxidizes linoleic & linolenic acids existing both as FFA's and monoglycerides, thus consuming the oxygen in the dough (Pyler, 1988).

Different sources of lipoxidase have different inactivation conditions (Zhou et al., 2007). The inactivation for wheat germ lipoxidase requires a very high temperature (Zhou et al., 2007). It remains active even after heating for 2 min at 95°C or after boiling for 3 min. It can also be inactivated by adding NaOH (Zhou et al., 2007). Heating between 110 to 140 °C reduced the percent of lipoxidase activity (Zhou et al., 2007). At 140 °C, wheat germ burned (Zhou et al., 2007). Heat inactivation of lipoxidase was successful at 10 min at 130 °C (Zhou et al., 2007).

The substrate for lipoxygenase is any FA with at least two *cis* double bonds interrupted by a methylene group (Gurr et al., 2002). Lipoxygenases work best on unesterified FA substrates, which are freed from storage TG's by lipases (Gurr et al., 2002). Linoleic acid and α-linolenic acid are substrates for plant lipoxygenase (Gurr et al., 2002). Because the lipoxygenase pathway involves free radicals, it can be inhibited by tocopherols or any other substances that trap radicals (Gurr et al., 2002).

Lipids are best isolated from flour and stored promptly in nitrogen under as low a temperature as possible, preferably -20°F or lower, to limit oxidation of double bonds (Gurr et al., 2002). Control of peroxidation is best done through reduction of the initiation step, through use of natural antioxidants, such as tocopherols, or synthetic antioxidants, such as 3,5-di-*t*-butyl-4-hydroxytoluene (BHT) (Gurr et al., 2002). Lipids should be dissolved in a small amount of solvent, and may have an antioxidant, such as BHT, added. Other substances used to control peroxidation include metal binding compounds, phenolic compounds, superoxide dismutase, and glucose oxidase-catalase systems. Isolation of lipids from plant sources must also be carried out quickly because many degradative enzymes retain activity even at very low temperatures, and may even be activated by dissolving in organic solvents (Gurr et al., 2002). These enzymes may be inactivated by boiling or steam treatment, or by extraction with hot isopropanol (Gurr et al., 2002).

Flour Lipid Extraction and Characterization

Lipid extraction is carried out by class and uses differential solubility to proceed from a crude extract to one with fewer components. Thus, most lipids require multiple solvents for

efficient extraction and isolation (Gurr et al., 2002). Binary solvent mixtures are often used where one solvent has some water-solubility and the ability to hydrogen bond, in order to split lipid-protein complexes. A number of different solvents are used to extract and separate lipids. Chloroform dissolves lipids, methanol dissolves non-lipid impurities, and solutions made of salt or dilute acid dissolve polar lipids (Gurr et al., 2002). All this has spurred the development of a number of different methods to extract specific lipids from flour.

Current lipid extraction methods are usually based on the original method of Bligh and Dryer (Gurr et al., 2002). In the Bligh and Dryer method, a choloform/methanol/sample water solution creates one phase in the lipid solution. Chloroform and methanol are then added to create two phases. The upper phase is aqueous and contains non-lipids, and the lower phase of chloroform contains lipids. The lower phase is then removed, washed with upper phase solution, and dried with nitrogen. Excess water is removed with anhydrous sodium sulphate or Sephadex column filtration. Salt or dilute acid solutions are employed in the upper phase to preserve polar lipids. Because the Bligh and Dryer method, more detailed lipid extraction solvent systems have been designed to employ more polar solvents, which may possibly extract more contaminants, such as amino acids, sugars, salts (Nichols, 2003). Much research on lipid extraction was done in the 1940's-60's, and was the basis for the later development of automated lipid extraction instruments (Nichols, 2003).

As an example of how sequential extraction is used to separate lipids, De Stefanis and Ponte (1976) used ethanol-benzene to extract total lipids from flour, benzene to separate the nonpolar from the polar lipids, and an aqueous methanolic solution to separate soluble from insoluble materials. Heptane was added to the solubles to separate diglycerides from free fatty acids while an acetone solution was added to the insolubles to separate triglycerides from steryl esters.

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is useful as a rapid, sensitive, and inexpensive method-with good resolution that requires minimal lab apparatus for lipid separations (Gurr et al., 2002). It is used for confirmation of the structure of an unknown substance using the relative retardation factor (R_f) (Gurr et al., 2002). It utilizes a stationary phase (silica on a glass plate) and a mobile phase (the solvent), which travels up the porous substance (silica gel) via capillary action (Gurr

et al., 2002) taking the lipids with it. Following the solvent separation of lipids, the plate is dried, sprayed with destructive solvent (dilute sulfuric acid) and heated, or sprayed with non-destructive (dichloro- or dibromo-fluorescein) fluorescent solvent and viewed (Gurr et al., 2002). Two-dimensional development of plates using two different solvent systems can be used to bring about further separation of components (Gurr et al., 2002).

In one example that studied flour lipids using TLC, nonpolar lipids in commercial baker's patent flour were classified by TLC using benzene-acetic acid-water as a solvent system, and the plate sprayed with sulfuric acid solution and heated (De Stefanis et al., 1976) for visualization. The method used for this study (see Materials and Methods section) is from Greenblatt et al (1995). It used a three-part process: extraction of free and bound lipids from the flour, elution of glycolipids, neutral lipids, and phospholipids, and further separation of lipid classes using one-dimensional TLC with specific solvent systems.

Baking Quality Research

Flour Quality

A definition of good flour quality encompasses a number of physical & chemical dough properties, depends on the end use of the flour, and is somewhat subjective (such as the distinction between "weak" and "strong" flours) (Pyler, 1988). According to Finney (1978), " a flour of good quality for bread-baking should have high water absorption, a medium to medium-long mixing requirement, satisfactory mixing tolerance, and good loaf volume potential (considering protein content), and should yield a loaf with good internal crumb grain and color." Other flour quality factors are: makes good bread over a wide range of processing conditions, able to carry protein-diluting ingredients or weak flour, not too long mixing time, makes a well-shaped loaf with high specific volume, and fine, resilient crumb structure (Pyler, 1988). The two components of gluten are also responsible for flour quality (Pyler, 1988). The gliadin fraction is responsible for loaf volume, while the glutenin fraction is responsible for dough mixing time and dough development (Pyler, 1988). Pomeranz (1988) lists the following as components of flour quality: protein quantity and quality, minerals, color, moisture, absorption, viscosity, enzymatic activity, granulation (particle size) and grain hardness, starch damage, response to additives, nutritional fortification, rheological properties, and bake tests for quality attributes. He defines flour quality as "the ability of the flour to produce a uniformly good end product under

conditions agreed to by the supplier and the customer." Baking quality can be evaluated using an optimized baking test to measure loaf volume and crumb grain potential (Finney, 1978).

A number of methods are employed to test the functionality and quality of flour, including baking tests, physical dough tests, and chemical analyses (Sluimer, 2005). Physical dough tests include the Farinograph, the Extensigraph, and the Alveograph (Sluimer, 2005). Chemical analyses include moisture, protein quality and quantity, ash content, and Falling number (Sluimer, 2005).

Post-Harvest and Milling Changes in Wheat

Posner et al (1997) described post-harvest changes in wheat. "Sweating" is comprised of the time between harvest and milling, and involves post-harvest changes in wheat. A number of options are available for millers and bakers to adapt to the new crop wheat flour, such as the addition of 5-15% new wheat to old wheat, having a set time period after harvest to completely switch over to new wheat (~3 months), or storing 2-3 months and then gradually incorporating the new crop flour. It is typical to blend old flour with new flour to damper the effects of new crop phenomena. Directly after milling, flour has a fine particle size, then an increase in particle size during the "sweat," and then a decrease in particle size after 19 weeks. Sweating is claimed to cause an "agglomeration of starch granules and interstitial proteins" (Posner et al., 1986) in endosperm. Therefore, larger chunks of endosperm are milled as sweating continues. Another change that can occur during wheat storage is that FFA's, MG's, and DG's increase slightly after the wheat has ripened (Morrison, 1978b). According to Posner et al (1986), after 14 weeks, the cost of flour storage outweighs any additional beneficial post-harvest changes to flour.

Posner et al (1986) conducted a study in which flour was baked directly after milling, and again 3 weeks later. No significant changes in absorption, mixing time, or loaf vol. were found between the fresh flour and the 3-week-old flour. However, subjective scoring showed that 3 weeks flour storage gave improved bread characteristics, specifically, a more tender crust, more uniform crumb grain, and improved loaf appearance and color. Loaf volume peaked at 112 days after storage; which is close to the value of 120 days found by Shellenberger (1939) in a similar study (Posner et al., 1986).

Whole Wheat Milling

The milling process of whole wheat flour is similar to that of refined flour. However, for whole wheat flour, a portion of the germ may be removed to prolong shelf life. The bran fraction is separated from the endosperm fraction, is re-ground to decrease particle size, and then is re-combined with the endosperm fraction to comprise the final flour. Whole wheat flour properties are more variable than those of white flour (Gan et al., 1989). Particle size ranges of whole wheat flours can be quite variable, depending on the mill and the milling process used. As described in the following section, "Components of Whole Wheat Flour," there are a lot of stability issues in whole wheat flour due to the high lipid levels.

Effects of Baking with Components of Whole Wheat Flour

Protein dilution by bran and germ is not the only reason for decreased bread volume of whole wheat bread (Gan et al., 1989). As visualized with Scanning Electron Microscopy (SEM), non-endosperm components, especially the epicarp hairs, disrupt the gluten-protein matrix (Gan et al., 1989). Bread made from whole wheat flour will have coarse or dense crumb, thick gas-cell walls and a discontinuous gluten-protein matrix, and low specific volume (Gan et al., 1989). If extra gluten is added, the cell walls become thicker, smoother, and more continuous (Gan et al., 1989).

Bran

In wheat flour milling, the pericarp and aleurone are separated from the remaining components and then further separated into bran and shorts (Lai et al., 1989a). In dough, wheat bran increases absorption, reduces mix time, decreases dough mixing tolerance, decreases dough strength, and reduces loaf volume (Zhang et al., 1997). These effects are amplified with increasing levels of bran added to the dough. In addition, wheat bran causes a virtually linear decrease in loaf volume with increase in bran amount (Lai et al., 1989a). There is also a linear relationship between protein and loaf volume (Lai et al., 1989a). However, the negative effects of bran on breadmaking are not just from the dilution of the gluten proteins. There are other chemical factors which affect bread-making (Lai et al., 1989a).

Bran is quite variable (Lai et al., 1989a). Its properties depend on the mill itself, the milling process, variety of wheat, and other factors (Lai et al., 1989a). During milling, there is a

varying degree of separation of bran from germ, which results in variable effects on baking (Lai et al., 1989a). Bran can have positive or negative effects on baking performance, depending upon how the bran sample is prepared (Zhang et al., 1997). Finer bran may cause a reduced mixing time and increased mixing tolerance, but has no effect on absorption (Zhang et al., 1997). The negative effects of bran can be counteracted with increased absorption, addition of shortening and SSL, fine grinding bran, and/or pre-soaking the bran (Lai et al., 1989a).

Pomeranz et al (1977) found that bread can be baked with up to 15 % of flour replaced by wheat bran. At this level, there was an increased absorption of 4 %. The addition of that level of bran caused no consistent effect on mixing time. Loaf volume decreased to the level expected from the dilution of gluten proteins. Once the added bran level was greater than 7 %, loaf volume decreased much more than expected, due to decreased gas retention. The incorporation of wheat bran caused decreased softness of bread and changed the bread color.

Wheat dietary fiber alters the quality and sensory properties of bread (Gomez et al., 2003). Fiber changes color, flavor, and odor of bread. A level of 2% fiber can be added to white bread without a decrease in bread palatability, while a level of 5% fiber requires additives such as vital wheat gluten (VWG), oxidizers, and emulsifiers to correct for changes to rheological properties of dough. In dough, fiber causes higher absorption, increased mixing tolerance, and decreased extensibility (Gomez et al., 2003). Increased absorption is due to hydroxyl groups in the fiber which allow more water interactions with water via hydrogen bonds. Fiber causes thickening of the walls which constitute air bubbles in the crumb. Fiber enhances the shelf life of bread, possibly by binding with starch to decrease the firming rate of bread (Gomez et al., 2003).

Shorts

Shorts consist of germ, aleurone, and pericarp layers, are contaminated with endosperm, and contain more aleurone and germ than bran does (Lai et al., 1989b). Shorts have a detrimental effect on loaf volume, due to the interaction between lipoxygenase, glutathione, and methoxyl hydroquinone (Lai et al., 1989b). A modification to the breadmaking process allows shorts to be added without the detrimental effects (Lai et al., 1989b). This modification involves either adding lipoxygenase in a no-yeast sponge or activating endogenous lipoxygenase by

soaking the shorts in water (Lai et al., 1989b). Shorts have a more detrimental effect on dough mixing properties and bread volume than does bran (Zhang et al., 1997).

Baking Studies on Flour Lipid Exchange

Lipids in refined wheat flour do not immediately degrade when the flour is stored at 59-95 °F, but they do undergo slow hydrolysis (Pyler, 1988). When flour lipids are stored in a high moisture environment, the lipids degrade and that affects baking quality. Flour lipid degradation produces several negative effects, including, doughs which lack extensibility, tear easily, retain gas poorly, and yield bread with poor volume, flavor, and palatability. These factors have been established by lipid exchange studies (Pyler, 1988).

A number of studies have examined the effects of lipids on baking quality. Using a lipid exchange approach, Barnes and Lowy (1986) determined that both the extracted lipids and the unextracted flour residue were responsible for causing decreased baking quality. Another lipid exchange experiment conducted by Larsen et al. (1989) did *not* show that high levels of non-polar lipid levels produced decreased loaf volume.

Hoseney et al (1969) conducted a lipid reconstitution experiment. They found that, when added to defatted flour, small amounts of free polar or nonpolar lipids were detrimental to loaf volume unless combined with native nonpolar or bound lipids. However, large amounts of free polar or nonpolar lipids recovered loaf volume. When added to defatted flour, nonpolar lipids either alone or combined with native bound polar lipids, caused no effect on loaf volume to bread that contained shortening. Also, when added to defatted flour, large amounts of bound polar or free polar lipids restored loaf volume, but higher levels of bound than free lipids were necessary.

Principles of Flour Storage Changes

Intact wheat undergoes a series of biochemical changes after harvest and during storage. Industrially, this is known as the ‘sweat.’ Flour storage biochemical reactions are very complex (Pyler, 1988). A post-milling maturation of ~5 days gives acceptable bread, but with limited dough elasticity (Pyler, 1988). Sweating/respiration is a necessary process for flour aging, which causes beneficial biochemical and oxidative changes in flour (Pyler, 1988). Sweating occurs from 5 days post-milling to 3 wks post-milling. During this time the flour is not as good for breadmaking (Pyler, 1988). Ideal storage conditions for flour are a light, well-aerated room at

constant temp of 75-80 °F (24-27 °C) (Pyler, 1988). Flours lose moisture if they are stored at <60% humidity (Pyler, 1988). Insect/fungal/bacterial activity occurs during storage if flour has a moisture content >12% (Pyler, 1988). The greatest moisture variation is in flour stored in paper sacks vs. in bulk trucks/railcars (Pyler, 1988). Flour stored in the presence of air drops in pH, while flour stored under N_2 maintains a constant pH (Pyler, 1988). The rate of change of flour parameters is considerably reduced at storage temps of 32 °F to -22 °F (0° to -30 °C) (Pomeranz et al, 1970). Flour stored at low temperatures gives poor baking quality; this may be remedied by moving the flour to a warmer storage location for 1-2 days prior to baking (Pyler, 1988). Flour storage results in the oxidation of flour lipids, carotenoids, & thiol groups of flour proteins (Pyler, 1988). During flour storage, the modification of lipids which are bound to gluten and the formation of FFA's change the quality of the gluten (Pyler, 1988). FFA's can increase from 5% to 70% of flour lipids during the flour aging process (Morrison, 1978b).

Flour Storage Studies

In addition to lipid exchange experiments, flour storage studies have also been conducted with bread made using white flour or whole wheat flour. Shelke et al (1992a) found that soft wheat (cake) flour undergoes beneficial changes with storage. During cake flour treatment, chlorine attacks most of the unsaturated FA's in nonstarch lipids, and has no effect on starch lipids (Morrison, 1978b). Shukhnov et al (1982) compared container storage vs. bulk storage for two different flours (termed 1st grade and 2nd grade) and found that the biological processes occurring during storage are similar in container-stored flour to those in bulk-stored flour. Exclusion of flour from interaction with outside air over time causes a build-up of volatile compounds which give a stale odor to flour. Flour storage caused only a slight change in flour total fat content but a notable increase in fractions of free fatty acids and a notable decrease in the triglyceride fractions. The decrease in FFAs and increase in TGs was less in the 2nd grade flour, apparently due to the effect of antioxidants naturally present in the flour (Shukhnov et al., 1982). The same authors conducted a summer flour storage experiment, during which time the total content of unsaturated fatty acids decreased (oleic, linoleic, and linolenic) and the total content of saturated fatty acids increased (palmitic and stearic), with the largest change being the decrease in linoleic acid. Lipase activity rose from storage day 5-15 for the 1st grade flour and from day 7-12 for the 2nd grade flour; after these periods it then decreased for each flour. The 2nd

grade flour had a higher lipoxygenase activity. Lipoxygenase activity increased for 1st grade flour from storage day 1-10, and for the 2nd grade flour from day 1-5; after these periods the activity then decreased for each flour. Aging took 5-7 days for the 1st grade flour, and 2-3 days for the 2nd grade flour. The authors concluded that flour can be stored in bulk in a silo for 3 months for 1st grade flour, and 1.5 months for 2nd grade flour.

Warwick et al (1979) conducted a five-year storage study of three white flours, finding an increase in FFA's, a decrease in TG's, and regarding specific molecular species, a decrease in each of monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG), phosphatidyl choline (PC), *N*-Acylphosphotidyl ethanolamine (NAPE), and phosphatidyl ethanolamine (PE). TG's were hydrolyzed completely to FFA's, with no change to DG content or accumulation of monoglycerides. A drop in total fatty acids occurred, involving minimal changes in amounts of palmitic, stearic, and oleic acid, and a large drop in polyenoic acids, especially linoleic acid. There was a decrease in both MGDG and DGDG, and an increase in their mono-acyl derivatives, MGMG and DGMG, which were hydrolyzed into FFA's and water-soluble components. NAPE, PC, and PE were each hydrolyzed to produce FFA's and water-soluble components. These changes were attributed to a combination of lipoxygenase action and autoxidation. In a follow-up study, Warwick et al (1980) further analyzed the three flours and found in the FFA fraction of the stored flour, monohydroxy-octadecadienoic acid methyl ester (MHA) and dihydroxy-octadecadienoic acid methyl ester (DHA), which are known to be products of lipoxygenase action.

Recent Industry Use of Whole Wheat Flour

Whole wheat flour is very important for health. It contains a variety of traditional and non-traditional healthful ingredients, such as dietary fiber, vitamins, minerals, phytochemicals, and antioxidants (Slavin et al., 2001). It can decrease the risk of coronary heart disease, cancer, and diabetes; lower serum and LDL cholesterol, improve colon function, and decrease the need for oxidative defense mechanisms (Slavin et al., 2001).

There has been a 20% increase in whole grain consumption since 1998 (Milling & Baking News, 2009). This includes whole wheat bakery products, such as whole wheat bread. These systems are high in lipid, which is present mainly in the germ fraction. Whole wheat flour

has been known to be variable in quality, due to flour age. In bread products, increased flour age is assumed to cause a decrease in flour quality, and thus, bread quality.

Gaps in Previous Research

From the previous research discussion, it is clear that several gaps still exist in our knowledge of the baking quality of whole wheat flour. Baking quality is very complex and involves many synergistic reactions and processes. This problem is compounded by the fact that there is less information available on the baking quality of whole wheat flours compared with information on lower extraction, refined, flours.

The objectives of this present investigation were:

1. Develop a standardized pup loaf baking method for whole wheat bread
2. Develop a laboratory scale flour milling procedure to produce a whole wheat flour similar in particle size distribution to a commercial sample
3. Test the effects of flour age post-milling on whole wheat bread quality
4. Determine what, if any, relationship exists between whole wheat quality and the flour glycolipid fraction

The hypothesis is that there are changes in the bread baking quality of whole wheat during the post-milling room temperature storage, and that changes in the lipid portion of the whole wheat flour are responsible for creating a detrimental effect on bread baking quality.

CHAPTER 2 - Materials and Methods

Materials

Flour

Moisture and protein analyses of flours were carried out in the analytical laboratory of the Dept. of Grain Science at Kansas State University, using methods 44-15A and 46-30 (AACCI, 2000). Four different whole wheat flours (Table 2.1), aged at room temperature for approximately one year, were the gift of Dr. Jeff Gwirtz (KSU Dept. of Grain Science). They were used to test whole wheat mixing properties and for baking test development. Flour properties: Aged Whole Wheat Samples for Baking Quality Data Generation

Table 2.1 Flour properties: Aged whole wheat samples for baking quality data generation

Flour	Protein % (14 % m.b.)	Moisture %
Hudson Cream 100 % Hard White Whole Wheat	12.9	8.5
King Arthur All-Natural Hard White Whole Wheat	12.7	8.7
King Arthur All-Natural Traditional Hard Red Whole Wheat	13.9	8.8
Untempered Whole Wheat (KSU roller mill)	11.8	10.4

The refined flour baking standard (Table 2.2) was donated by ADM Milling Company, North Kansas City, MO. The hard red winter whole wheat flour (WWF) (Table 2.2) used as the whole wheat control flour, was a commercial composite. It was donated by General Mills (Minneapolis, MN).

Table 2.2 Refined flour and WW fresh flours used as baking standards for WW flour test baking method development

Flour	Protein % (14 % m.b.)	Moisture %
Polar Bear (PB) Refined flour: composite HRW of hard winter wheat flours	10.8	13.1
Whole wheat flour (WWF): commercial composite HRW	13.6	12.2

Hard red winter whole wheat flour (Karl 92, harvested 2006) (Table 2.3) was obtained from the Kansas Seed Foundation, and milled into whole wheat flour in the Dept. of Grain Science (see Methods).

Table 2.3 Fresh WW flour milled specifically for WW test baking research

Flour	Protein % (14 % m.b.)	Moisture %
HRW WWF (Karl 92)	15.33 %	12.37 %

Baking Ingredients

Shortening used was Crisco® All-Vegetable Shortening, (J.M. Smucker Company, Orrville, OH). It contains soybean oil, fully hydrogenated cottonseed oil, partially hydrogenated cottonseed and soybean oils, mono and diglycerides, TBHQ and citric acid (antioxidants). L(+)-Ascorbic acid, ACS reagent grade, was obtained from Acros Organics (Morris Plains, NJ). Instant Active Dry Yeast was from Saf-Instant®, manufactured for Lesaffre Yeast Corporation (Milwaukee, WI). Malted Barley Flour was obtained from ADM Milling Co. (Decatur, IL).

Lipid Extraction & Chromatography Materials & Chemicals

Silica Plates for TLC

- Precoated TLC Glass Plates, Silica Gel 60; (20 x 20 cm); 0.25 mm layer thickness, 25/ pk; EMD Chemicals, Darmstadt, Germany; Item number 5721/7

Solvents

The following, all reagent grade, were purchased from Sigma-Aldrich Co., St. Louis, MO, USA:

- Acetic acid, 1L; CAS #64-19-7; Item #320099
- Acetone, 1L; CAS #67-64-1; Item #179973
- Acetonitrile, 1L; CAS #75-05-8; Items #110086 and #270717 (the latter was since replaced by item # 34851)
- Ammonium hydroxide solution, 2.5L; CAS #1336-21-6; Item #320145
- Chloroform, 4L; CAS #67-66-3; Item #132950
- (Di)Ethyl ether, 1L; CAS #60-29-7 ; Item #673811
- Ethanol, 2L; CAS # 64-17-5; Item #277649
- Ethyl acetate, 1L; CAS # 141-78-6; Item #320307
- Hexane, 4L; CAS #110-54-3; Item #208752
- Hydrochloric acid, 2.5L; CAS #7647-01-0; Item #258148
- Isopropanol, 4L; CAS #67-63-0; Item #109827
- Methanol, 4L; CAS #67-56-1; Item #179337
- Sulfuric acid, 2.5L; CAS #7664-93-9; Item #320501
- Toluene, 1L; CAS #108-88-3; Item #179965
- Tetrahydrofuran, 1L; CAS #109-99-9; Item #178810

The following, all reagent grade, were purchased from Acros Organics, Morris Plains, NJ, USA:

- Hydrochloric acid, 2.5L; CAS #7647-01-0; Item # 124630025

The following, all reagent grade, were purchased from Fisher Scientific, Pittsburgh, PA,USA:

- Isopropanol, 4L; CAS #67-63-0; Item #A451-4
- Methanol, 4L; CAS #67-56-1; Item #A452-4
- Petroleum ether, 4L; CAS #8032-32-4; Item #E139-4

Other

- Nitrogen gas, high purity, tanks; CAS no. 7727-37-9; Linweld Co., Waverly, NE, USA; Type: LW410

Lipid Standards

The following, all reagent grade, were purchased from Sigma-Aldrich Co., St. Louis, MO, USA:

- Glycolipids
  - DGDG (Digalactosyl diglyceride) from whole wheat flour, lyophilized powder, 1 mg; MDL #MFCD00133266; Item #D4651
  - MGDG ((Mono)Galactosyl diglyceride) from whole wheat flour, vacuum-dried, 1 mg; MDL #MFCD00213918; Item #G9523
- Phospholipid
  - L-α-Phosphatidylcholine, lyophilized powder, from soybean; 100 mg; CAS #8002-43-5; Item #P7443

The following, all reagent grade, were purchased from Supelco, Bellafonte, PA, USA:

- Neutral Lipids
  - Linoleic acid, 100 mg clear colorless liquid, from Fatty Acids, Unsaturated, Even Carbon Kit; Item #UN10-1KT
  - Linolenic acid, 100 mg clear colorless liquid, from Fatty Acids, Unsaturated, Even Carbon Kit; Item #UN10-1KT
  - Tripalmitan, 100 mg dry powder, from Mono-, Di-, and Triglycerides Kit; Item #MDT12-1KT

Methods

Wheat and Flour Analysis

The moisture of Karl 92 wheat was determined prior to milling using Method 44-15A (AACCI, 2000). Flour properties were determined for all flours, including protein content Method 46-30 (AACCI, 2000), moisture Method 44-15A (AACCI, 2000), falling number Method 56-81B (AACCI, 2000), mixogram curve Method 54-40A (AACCI, 2000), absorption, and mixing time. Unmalted flours were treated with malted barley flour. The falling number Method 56-81B (AACCI, 2000) was used to determine amylase supplementation levels in flour. Malt was added incrementally to reach the optimal amylase level of 250. Mixing time was determined by mixing flour at the optimized mixograph amount, and water at the optimized mixogram amount and at absorptions at increments higher and lower than the optimum mixograph absorption, after which 100 g loaves were baked to determine which absorption level was optimum for dough handling throughout the rest of the baking process.

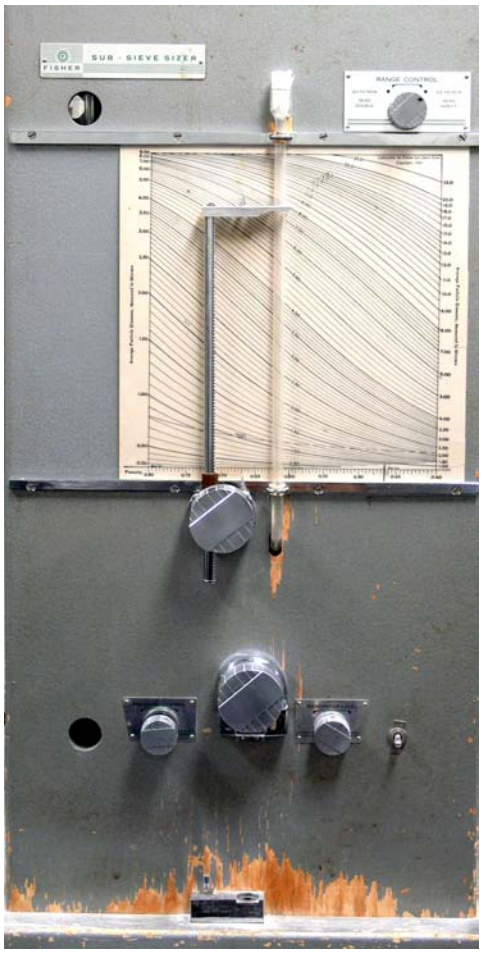
Granulation Curves

Granulation curves for the standard, commercial, and experimental whole wheat flours were produced using Ro-Tap Sieve Shaker (see Fig. 2.1) modified from Method 66-20 (AACCI, 2000) (Ro-Tap, The W.S. Tyler Co., Cleveland, OH, USA). Size or percent throughs was measured with the Alpine Air Jet Sieve (Fig. 2.2) (Alpine, Augsberg, Germany) (Hosokawa Alpine Aktiengesellschaft: Alpine operating instructions). Average particle size was measured using the Fisher Sub-Sieve Sizer (Fig. 2.3) (Fisher Scientific Bulletins 14-311 and 14-311V2). The Fischer Sub-Sieve Sizer predicts average particle size of flour (µm) by subjecting a specified weight of flour, packed in a column, to a stream of pressure-regulated air and measuring its porosity. Air moves more quickly through coarse particles then through fine particles. The column is connected to a water-filled manometer, in which the height of water corresponds to the flour's average particle size on a chart behind the column, which was calculated by Gooden et al (1940) based on work by Carman (1938).

Figure 2.1 Ro-Tap Sieve Shaker

Figure 2.2 Alpine Air Jet Sieve

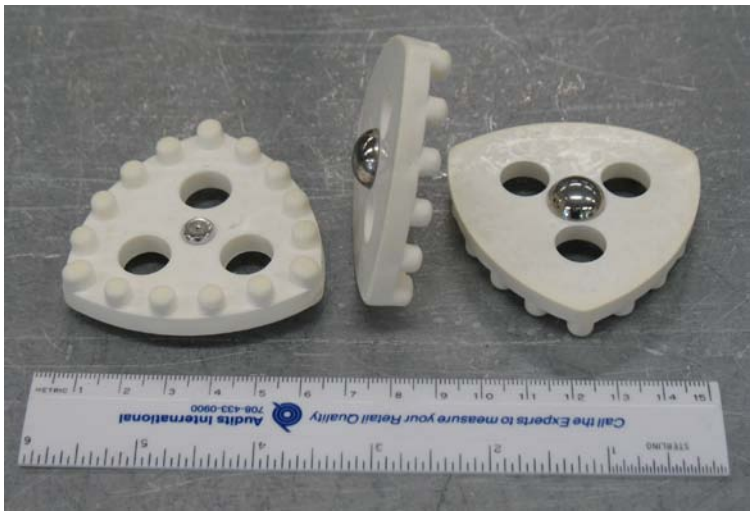
Figure 2.3 Fisher Sub-Sieve Sizer



The Ro-Tap test consisted of sifting 100.0 g (3.53 oz) of blended flour, for 6 minutes, with two plastic triangle pan cleaners per sieve (Sefar America, H.R. Williams Division, Kansas City, MO) (see Fig 2.4). The triangular plastic pan cleaners are 2 ½" wide by ¼" thick. Data was recorded as the overs of US Standard Sieves #20, 30, 40, 60, 100, and in the pan, at respective openings of 841, 594, 420, 250, 150, and <150 microns. Data were graphed of the cumulative percent over with respect to sieve screen opening sizes (Rozsa, 1948). Alpine Air Jet Sieve used a 25 g sample of blended flour, with sifting for 5 minutes. Results were calculated as the percent through the 200 US mesh sieve screen (75 µ). A sample of 1.44 g of the flour from the throughs of the 150 µm sieve of the Alpine test was then analyzed in the Fisher Sub-Sieve Sizer test. The Fisher Sub-Sieve Sizer test measures the average particle diameter of the flour, in Fisher Average Microns (Fisher Scientific Bulletins 14-311 and 14-311V2). Average particle

diameter of flour is based on the principle of air permeability and is determined using a multi-curve chart which is attached to the instrument. Average particle diameter is not affected by particle shape.

Figure 2.4 Plastic pan cleaners



Wheat Handling & Milling

Wheat, variety Karl 92, was harvested June 22, 2006. Following harvest, the wheat was stored in a bin, conditioned during July and August, and then stored in bags in an insulated warehouse at the KSU Seed Foundation in a range of 45-80 °F, at a moderate humidity. The wheat was obtained May 19, 2008 from the Kansas Foundation Seed Project Office of the KSU Dept. of Agronomy. Once the wheat was brought to the department, it was stored in its original bag in a -15 °F (-26 °C) freezer until milling day. It was then removed from the freezer, divided in half into two large clear plastic garbage-size bags, with the air expelled, tied shut, laid flat on the table, and thawed at room temperature until it reached room temperature (approx. 1-2 hrs.). Mill room conditions on Day 0 milling were 72 °F (22 °C) and 77 % RH. The wheat was not tempered prior to milling in order to be able to facilitate reducing the particle size of the bran during the milling process, thereby achieving WW flour with a uniform particle size.

The milling method (Fig 2.5) was specifically developed by Dr. Gwirtz so as to produce whole wheat flours that mimicked commercial WWF granulation curves. A single corrugated Ross experimental laboratory roller mill (Fig 2.6) (Ross Machine and Mill Supply, Inc.,

Oklahoma City, OK) in the Dept. of Grain Science was used to mill the wheat into flour. Only one corrugated roller mill stand was used for the entire milling process. No smooth rolls were used. The Ross Laboratory Roller Mill had a roll speed differential of 2.5:1, a pitch of 24/inch (9.4/cm), a spiral of ½ inch/ft (4.2 %), and a grinding action of dull:dull. See Fig 2.7 for a photo of the roller mill corrugations. The corrugation profile is a 24 Getchel.

Figure 2.5 WW Milling Method

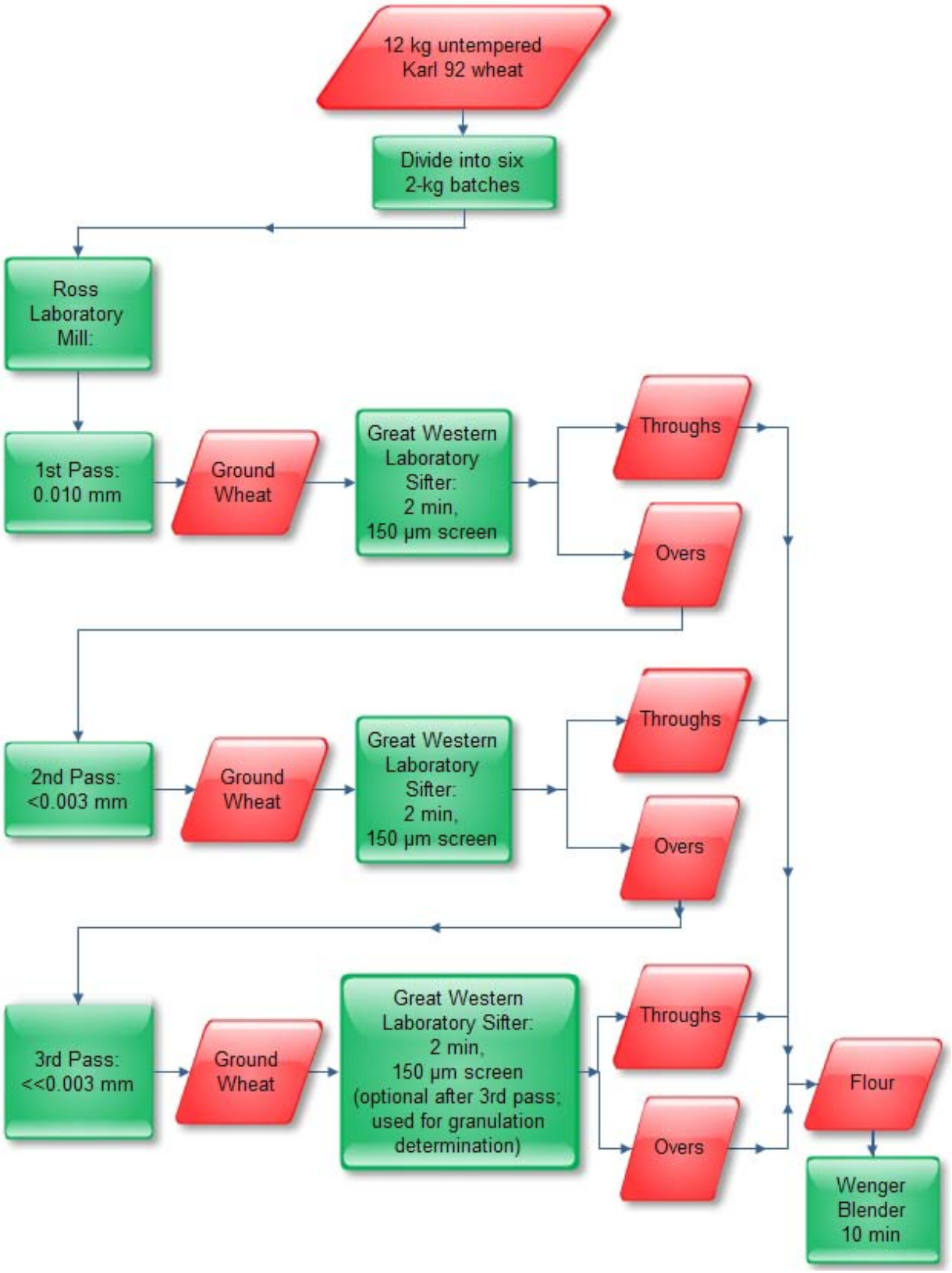
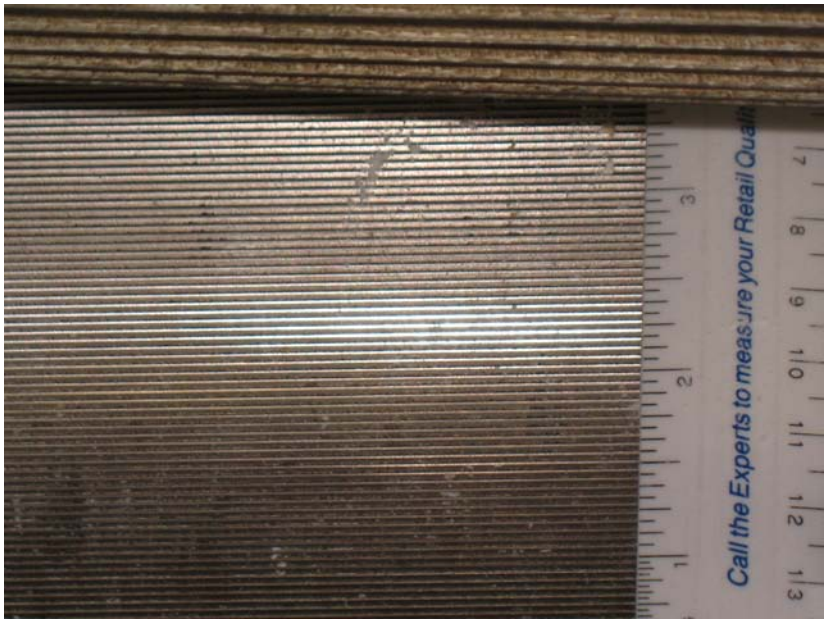


Figure 2.6 Ross Experimental Roller Mill

Figure 2.7 Ross Experimental Roller Mill Corrugations



The three-pass system was designed. The roll gap was set to 0.010 inch (0.0254 cm) using a feeler gauge for the first pass. Whole wheat flour was milled from 12,000 g of wheat, in batches of 2000 g, until all the wheat was all through the first pass. The second pass gap was set at <0.003 inch (<0.0076 cm). The third pass gap was set at <<0.003 inch (<<0.0076 cm); at as tight an opening as was possible without jamming the machine. The feed rate varied, but was 344.1 g/min ± 134.8 (0.7586 lb/min ± 0.2972) for 1st pass, 855.3 g/min ± 113.6 (1.8856 lb/min ± 0.2504) for 2nd pass, and 826.5 g/min ± 144.7 (1.8221 lb/min ± 0.3190) for 3rd pass. Between the 1st and 2nd passes and the 2nd and 3rd passes, the ground wheat was fed for 2 min with a 9XX 150 µm sieve through a Great Western Laboratory Sifter (Fig 2.8) (Great Western Mfg. Co., Leavenworth, KS, USA) with three plastic sieve cleaners. The throughs of the sieve were set aside as flour, and the overs (bran and unseparated bran-endosperm pieces) were collected to be run through the subsequent passes. After the 3rd pass, the throughs and overs were not run through the sieve shaker; both the throughs and overs were collected to be blended into flour. Once all of the wheat had been run through all three passes, the resulting flour was collected and blended for 10 min using a Wenger Double Ribbon Mixer (Fig. 2.9) (Sabetha, KS, USA), which has a capacity of ~175 lbs (~80 kg) flour to create one large batch. The resulting flour was left unbleached, and contained no additives. See Results for discussion on granulation curves and how the milling methods matched them.

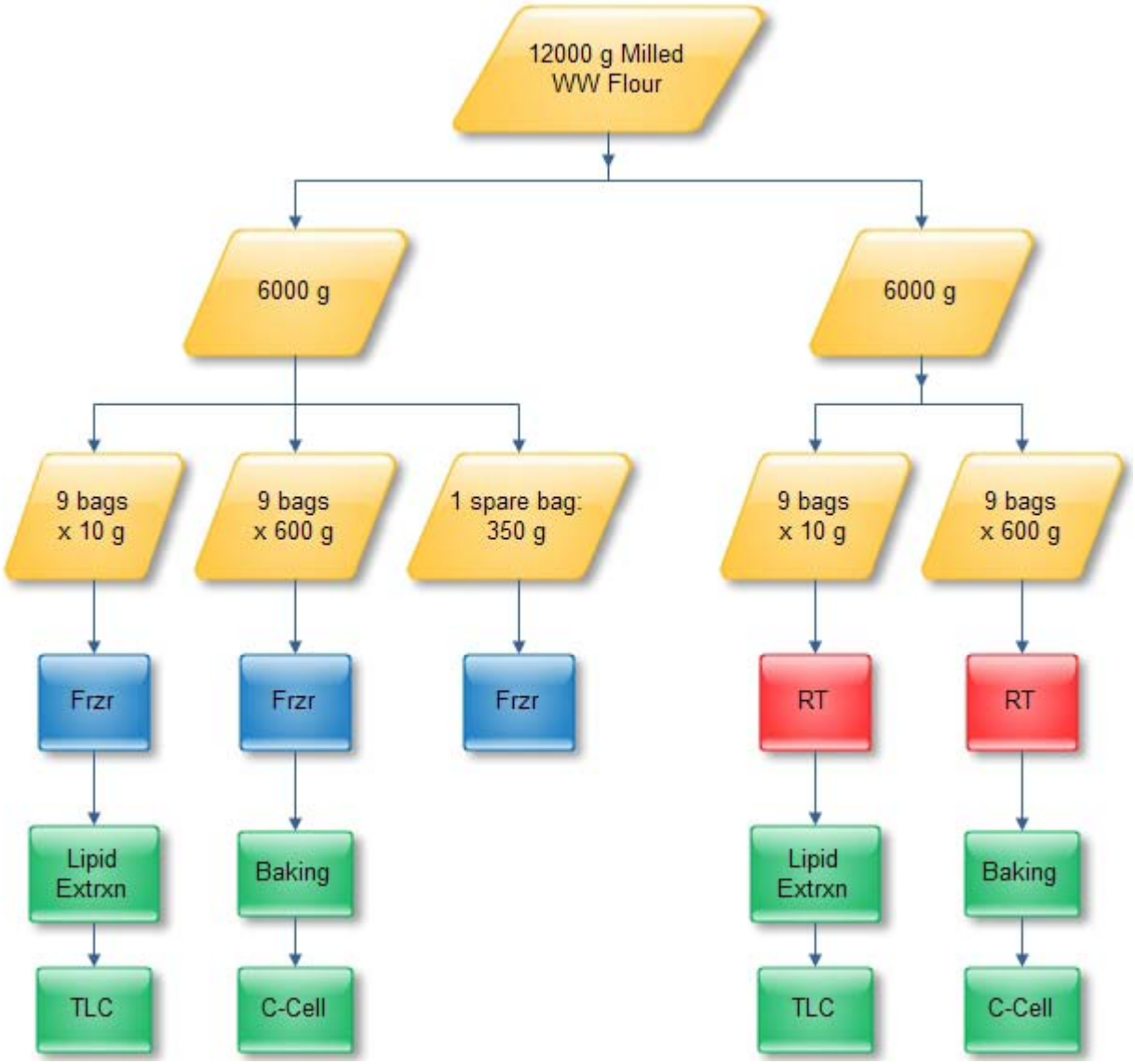
Figure 2.8 Great Western Sifter

Figure 2.9 Wenger Double Ribbon Mixer

Flour Storage

Of the 12,000 g whole wheat flour, 6,000 g was stored at deep freeze temperature of -15 °F (-26 °C) in the KSU Grain Science Bakery Lab freezer, and 6,000 g was stored, double plastic bagged, inside a plastic bin at constant room temperature of 72 °F (22 °C) in KSU Waters Annex 103B lab. See Fig 2.10 for the illustration of how the flour was divided and allotted. The flour for baking was bagged in 600 g lots (9 bags for Frzr, 9 bags for RT), while the flour for lipid analysis was bagged in separate bags of 10 g. One spare bag of 350 g of whole wheat flour was stored in the Frzr. At 3-day intervals over the 21 days after milling, a 600 g bag of flour was removed from each storage conditions (Frzr and RT) and brought to room temperature. Intact flours (unextracted) were baked in triplicate from each batch of flour on Days 0, 3, 6, 9, 12, 15, 18, and 21; Day 0 being the date of milling of the KSU flour. Bread loaf analysis included loaf height, loaf volume, loaf weight, and C-Cell images and data. Once baking was completed for the day, a 10 g bag was removed from Frzr and RT for lipid extraction. The Frzr flour was allowed to reach room temperature shortly before extraction. Flour lipids were extracted and analysis conducted using TLC.

Figure 2.10 Flour Storage and Purposes



Lipid Extraction and Thin Layer Chromatography (TLC)

Lipid Extraction Solutions (all solutions are v/v per Greenblatt et al. (1995)

1. Hexane, ethyl acetate, acetic acid (95:5:0.2)
2. Hexane, ethyl acetate (95:5)
3. Chloroform, methanol (2:1)
4. Tetrahydrofuran, acetonitrile, isopropanol (35:35:30)
5. Acetonitrile, methanol (35:65)
6. Isopropanol, water (90:10)
7. Hexane

TLC Development Solutions (all solutions are v/v) per Greenblatt et al. (1995)

A. Ethyl ether, toluene, ethanol, acetic acid (10:50:2:0.2)
B. Ethyl ether, hexane (6:94)
C. Chloroform, acetone, acetic acid, water (10:90:2:3)
D. Ethyl ether, acetic acid (99:1)
E. Chloroform, methanol, ammonium hydroxide, water (65:35:5:2.5)
F. Sulfuric acid, methanol (1:1)

Free and bound lipids were extracted from wheat flour by the method of Greenblatt et al. (1995). Lipids were extracted from 0.5 g flour with 5 ml hexane (Solution #7) by shaking for 1 hr at RT; with vortexing every 5 min. The tubes were then centrifuged at 3000 x g for 5 min. Supernatants were removed and saved for estimation of total free lipids. The remaining flour was dried under a dry nitrogen stream. Bound lipids were extracted from the pellet using 3.75 ml of 90 % propanol (Solution #6) by vortexing until suspended and then shaking for one hour (vortexing every 5 min) at RT. Tubes were again centrifuged at 3000 x g for 5 min. The supernatants were transferred to a new tube, and then dried under a nitrogen stream at RT. (Drying at 50 °C (122 °F) was suggested in the procedure, but was not employed here in order to limit lipid degradation due to excess heat.) The isolated free lipids, and bound lipids were separately dissolved in 2 ml of Solution #1, placed in tubes, flushed with nitrogen, sealed, and

stored at -15 °F (-26°C). The process for extracting free and bound lipids from flour is illustrated in Fig 2.11.

Bound and free lipids were then each separated into neutral, glyco-, and phospholipid fractions, as follows (see Fig 2.12). Silica Sep-Pak® cartridges were pre-washed with 5 ml of Solution #7 and then with 5 ml of Solution #1. Free or bound lipids were then applied to the Sep-Pak cartridge. Neutral lipids were eluted using 4 ml of Solution #1. The resulting eluted neutral lipids dissolved in Soln. #1 were then dried at RT under nitrogen, and then dissolved in 125 µL of Solution #3. In preparation for glycolipid extraction, 5 ml of Solution #2 was poured through the cartridge to wash it and then the cartridge was partially dried under nitrogen. Glycolipids were then eluted from the cartridge using 4 ml of Solution #4, and then dried at RT under nitrogen, and then dissolved in 125 µL of Solution #3. For phospholipid extraction, the cartridge was washed with 5 ml of Solution #2 and then the cartridge was partially dried under nitrogen. Phospholipids were eluted from the cartridge with 4 ml of Solution #5, dried at RT under nitrogen, and then dissolved in 125 ml of Solution #3.

Extracted lipids were stored in solution #3 (2:1 chloroform: methanol) in the -15 °F (-26 °C) freezer. Lipid standards included monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG), phosphatidyl choline, linoleic acid, linolenic acid (Sigma Chemicals). Each lipid standard was put in solution to reach a concentration of 10 µg/µL.

Figure 2.11 Extraction of Free and Bound Lipids

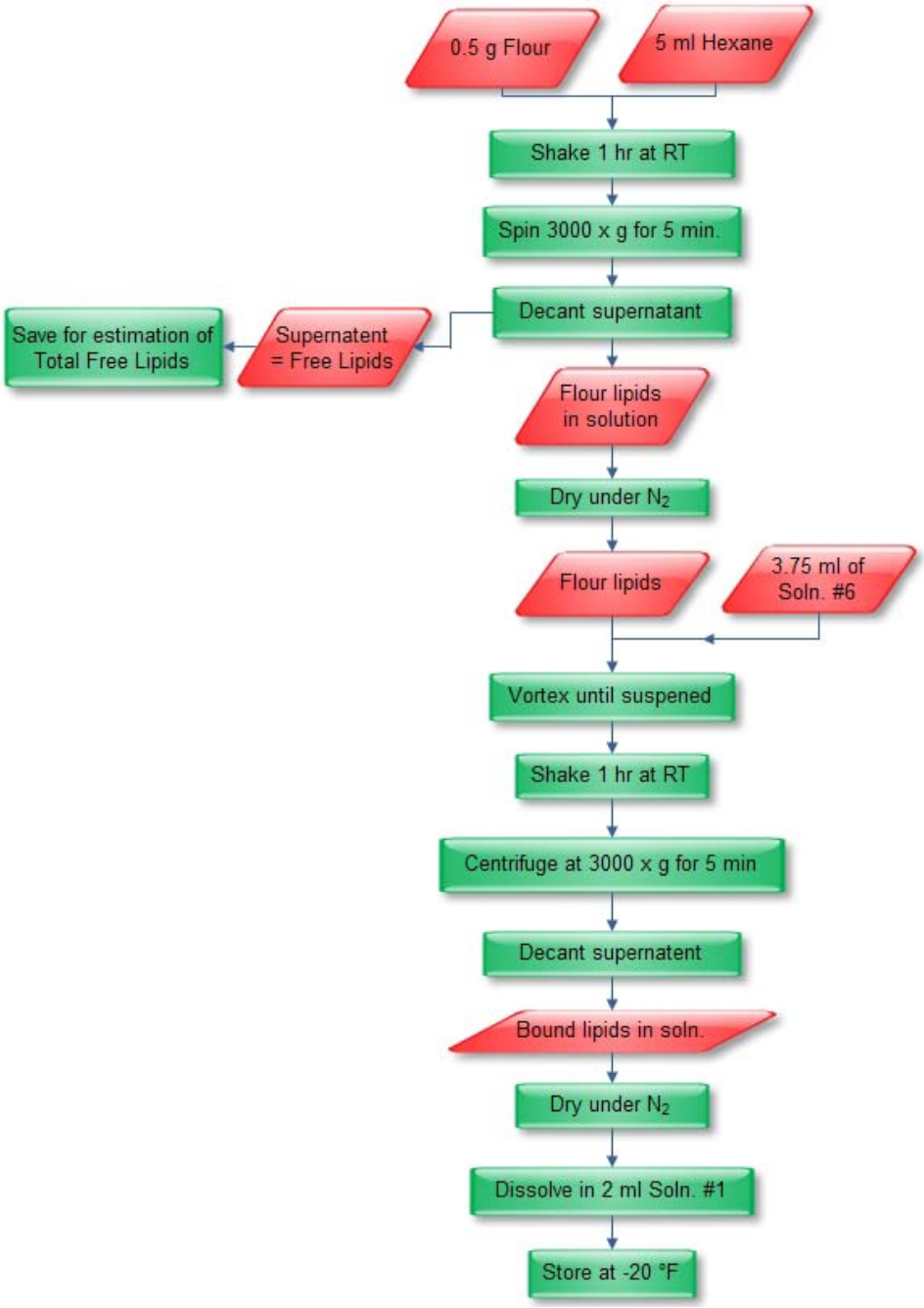
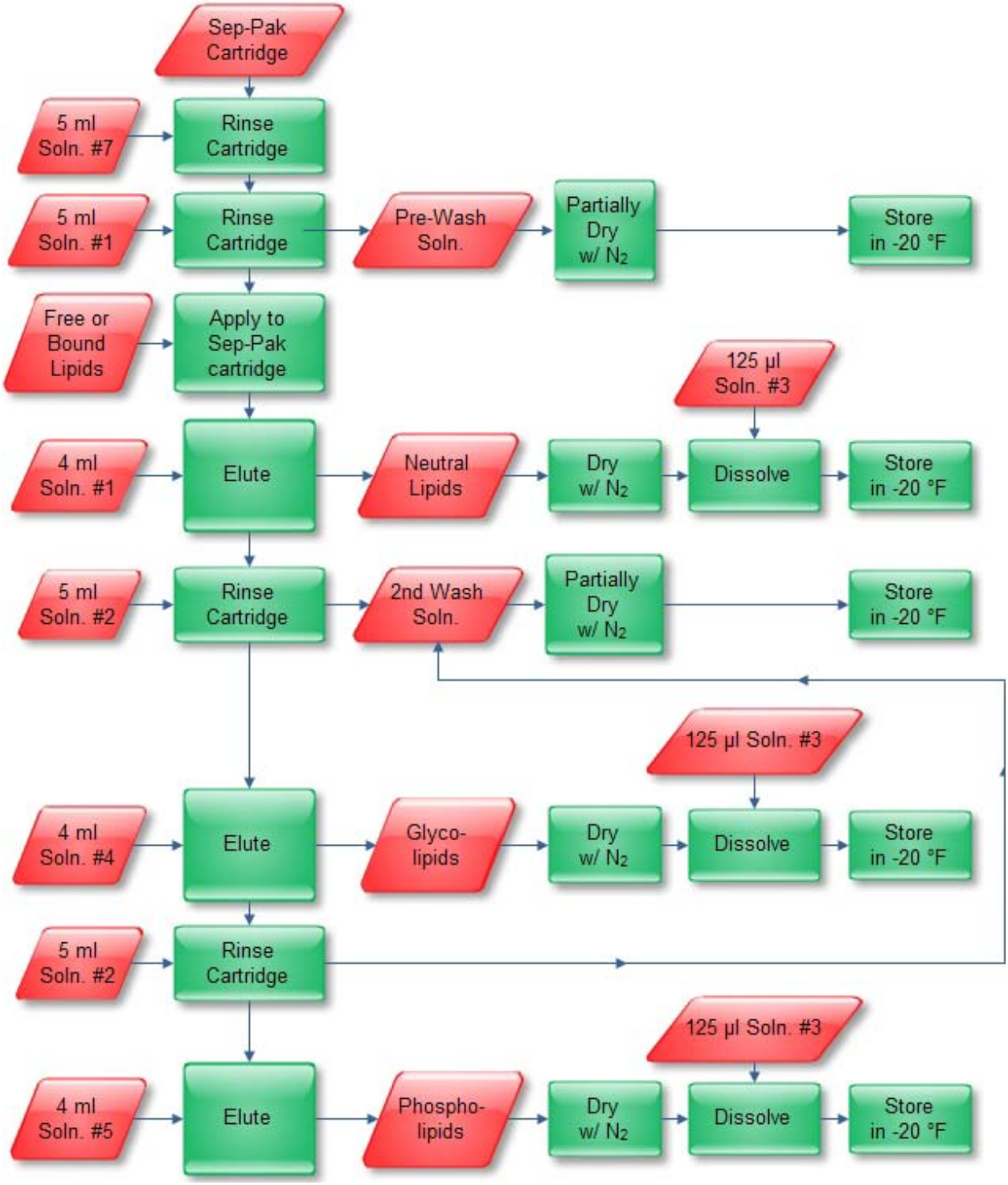
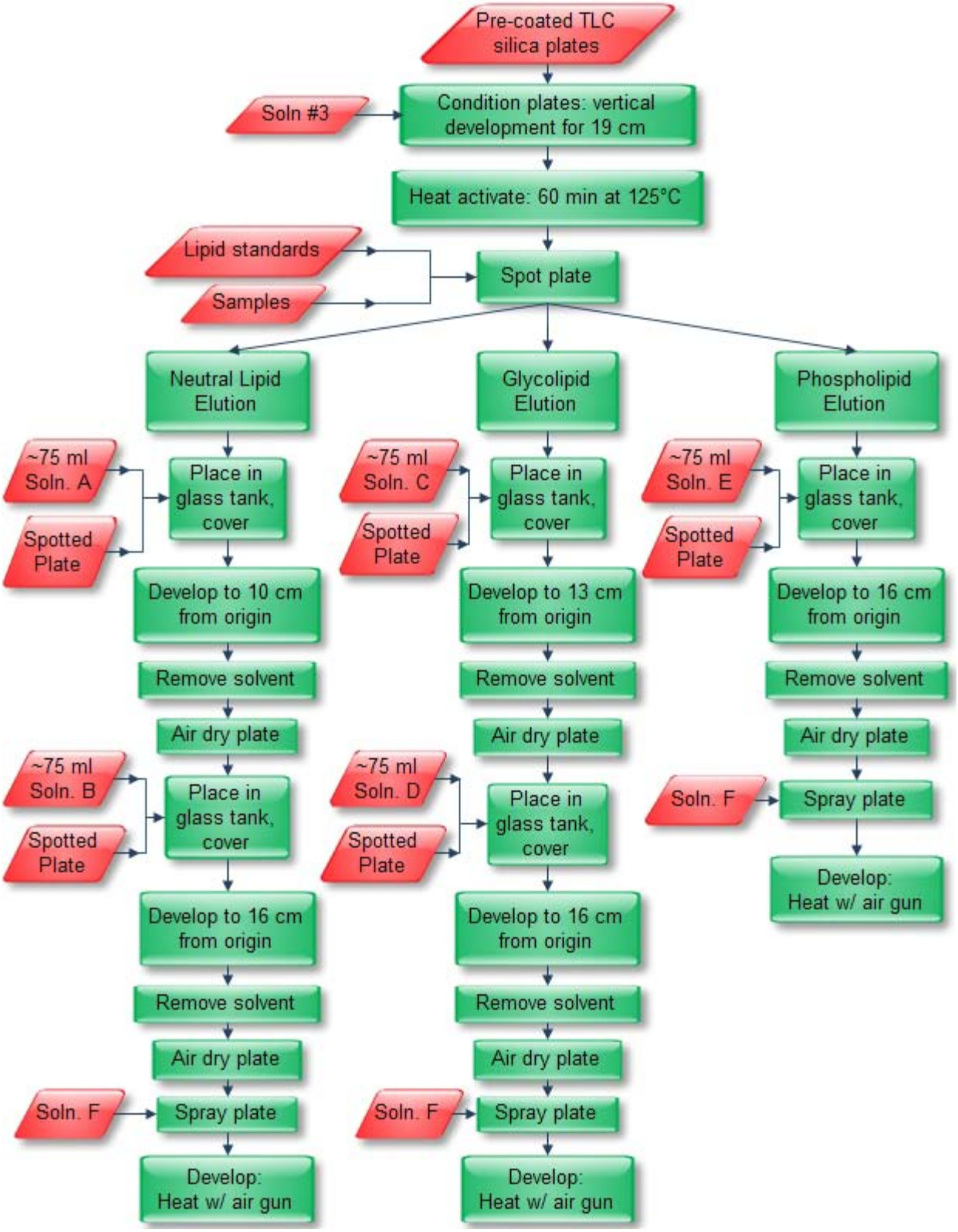


Figure 2.12 Extraction of Neutral lipids, Glycolipids, and Phospholipids



Thin layer chromatography (TLC) was used to isolate lipid fractions in flour from and to monitor lipid composition throughout the flour storage experiment (see Fig 2.13). Pre-coated 20 x 20 cm (7.9 x 7.9 in.) TLC silica gel plates (Greenblatt et al., 1995) were conditioned by vertical development in Solution #3 for 19 cm (7.5 in.). The plates were then heat-activated at 125 °C (257 °F) for ~60 min. Lipid solutions were spotted on the plate from 2 – 20 µL in 2 µL increments, allowing drying in between. Nineteen lanes were used per 20 cm (7.9 in.) wide plate, with 1 cm (0.4 in.) spacing between spots. Spots were placed 2 cm 0.8 in.) from the bottom of the plate. Tank solvent depth was maintained at 0.5 cm (0.2 in.), which required ~75 ml solution. Three neutral lipids, tripalmitan, linoleic acid, and linolenic acid, 1:1:1, with a succession of 0.6, 0.6, 1.5, 1.5, 2.1, 2.1, 3.0, 3.0, 3.6, 3.6 µL standard spots for each neutral lipid TLC plate were used as standards. Two glycolipid standards, MGDG and DGDG, 1:1, were combined to produce the glycolipid standard solution. A sequence of 1.0, 1.0, 1.4, 1.4, 2.0, 2.0, 2.4, 2.4, 3.0, 3.0 µL standard spots of glycolipid solution was used on each glycolipid TLC plate. One phospholipid standard, phosphatidylcholine, was used with a succession of 0.2, 0.2, 0.5, 0.5, 0.7, 0.7, 1.0, 1.0, 1.2, 1.2 µL standard spots, for each phospholipid TLC plate. A range was not suggested for neutral lipid standards, however appropriate ranges were 5 – 50 µg for glycolipid standards and 2 - 30 µg for phospholipid standards. Sample application amounts were; 5 µL for neutral lipid and glycolipid samples, 5 µL for free phospholipids and 10 µL for bound phospholipids. These levels were selected during preliminary testing so as to result in acceptable spot size and densities for Doc-It software to measure. Neutral lipids were separated using a two solvent system; first Solution A to 10 cm (3.9 in.) from origin, then Solution B to 16 cm (6.3 in.) from origin. Glycolipids were also separated using a two solvent system; Solution C to 13 cm (5.1 in.) from origin, then Solution D to 16 cm (6.3 in.) from origin. Phospholipids were separated with Solution E to a solvent front 16 cm (6.3 in.) from origin.

Figure 2.13 Thin Layer Chromatography Process



Once developed, TLC plates were air dried, sprayed with Solution F in a fine, even mist, and then developed by heating with an air gun. Plates were viewed and photographed with a 14.7 megapixel digital Canon Powershot G9 camera secured inside a MultiDoc-It^{TM} Digital Imaging System cabinet (UVP, LLC Upland, CA, USA) with a UVP Benchtop 2UV Transilluminator (Fig 2.14), which was connected to a computer. Photos of plates were then analyzed using Doc-It® software (UVP, LLC Upland, CA, USA).

2.14 MultiDoc-It™ Digital Imaging System Cabinet with Mounted Canon Powershot G9 Camera and UVP Benchtop 2UV Transilluminator

Breadbaking

Method 10-10b Straight Dough Bread baking method (AACCI, 2000) and formula was used as the basis of a whole wheat bread baking method. For development of the original method, see Finney (1984). A 90 minute fermentation was used, with the fermentation cabinet at 86 °F (30 °C) and 92-97 % RH. Each loaf contained 100 g of flour (14 % m.b.), 6 g of sucrose combined with 1.5 g of salt in an 11-ml aliquot with distilled water, 5 ml of 50 ppm ascorbic acid, 3 g of shortening, 2 g of instant active dry yeast, optimized water absorption level, and optimized malt level if unmalted flour. Initial water absorption was calculated based on the moisture and protein content of flour. Absorption and mixing time were further optimized using method 54-40A (AACCI, 2000) for the Mixograph (National Mfg. Co., Lincoln, NE) and a pre-baking test. The pre-baking test consisted of baking a series of loaves at incremental increases in absorption to confirm the most appropriate absorption. The optimized mixogram absorption plus a few absorptions lower and higher were evaluated during the baking process for mixing, dough handling, fermentation, proofing, sheeter/moulder handling, and baking characteristics. The preferred absorption level from the baking tests was neither too wet nor too dry throughout all bakery process stages, did not stick excessively to hands or to the sheeter molder, did not leave visible dry moulding creases after bake, and produced the largest loaf volume.

The whole wheat flour baking formula and process was adapted by modifying the water added based on changes in mixogram characteristics. In method 10-10b, water is added to flour in multiple ways; in the sugar/salt solution, and in combination with ascorbic acid (tall burette, filled to volume to reach the “mixogram minus 20 mls” number). For refined flour, the initial absorption was estimated by calculation (see results). The mixogram was then recorded, and the absorption value minus 20 was used to calculate the water to be added by the large burette. For whole wheat flour, a value of “mixogram minus 15 mls” was used. This was done because whole wheat flours continue to absorb water throughout the fermentation process, more so than refined flour does. Initial studies using WW Ctrl flour suggested that wheat flour needed additional proofing time to reach the 7.6 cm proof height. However, this was not the case for the Karl 92 flour milled specifically for the 21-day baking experiment.

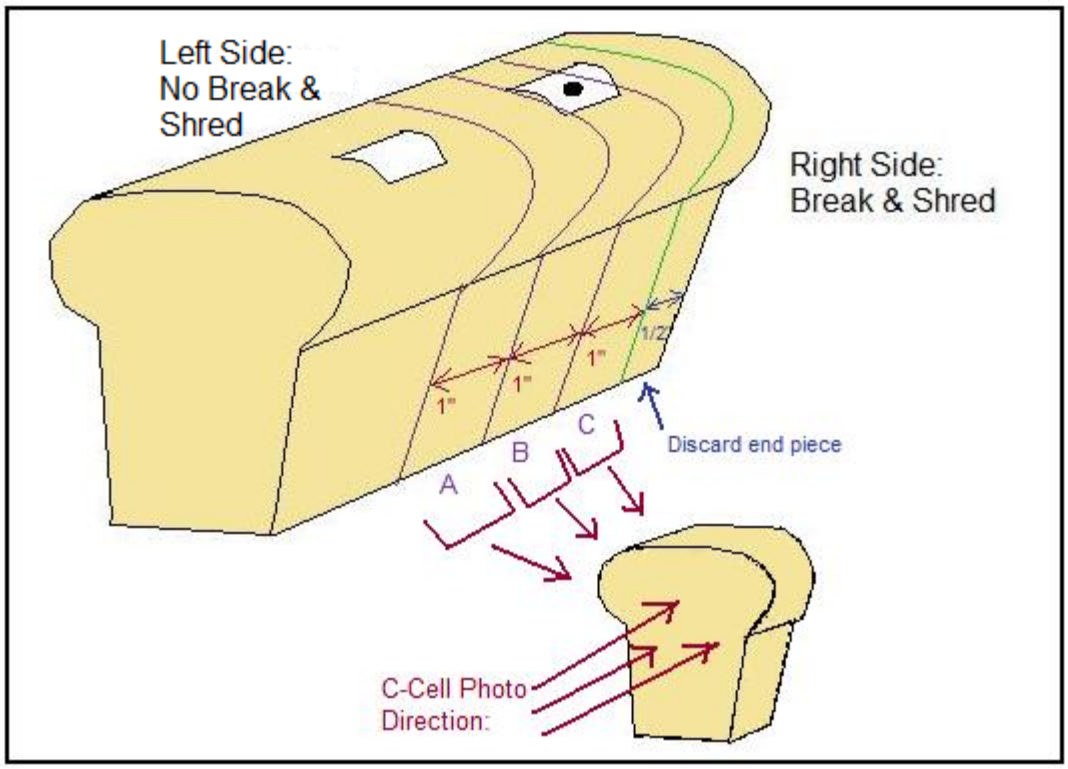
Fermenting doughs were punched at 52, 77, and 90 minutes, sheeted with automatic sheeter rolls (National Mfg. Co., Lincoln, NE), sheeted and molded using a Thomson Molder

(Thomson Machine Co., Belleville, NJ) and then panned. Dough was placed seam-side down and centered in pans, which were greased on bottom, one side, and ¼ of other side of pan. Doughs were then proofed to height of 7.6 cm (approximately 33 minutes proof) and baked 24 minutes at 215 °C (419 °F) in a reel oven (National Mfg. Co., Lincoln, NE). Loaf volumes were measured immediately after baking with rape seed displacement using Method 10-05 (AACCI, 2000). Loaf weights were recorded immediately after bake. Loaves were cooled 1 hr before being placed in sealed low density polyethylene bags to be stored at room temperature, which was approximately 22 °C (72 °F).

Digital Image Analysis (C-Cell)

Digital image analysis was carried out using the C-Cell (CCFRA Technology, Ltd., U.K.) with C-Cell Version 2.0 software. Loaves were prepared for image analysis by slicing with an electric serrated bread knife. The loaf was placed in a slotted bread slicing holder with break and shred side facing up. Then, a ½" (1.3 cm) wide slice was removed from the right side of the loaf. Three 1" (2.5 cm) slices were then cut from the direction of the sliced end, and images taken from the farthest 1" (2.5 cm) slice, with the break and shred placed to the right (Fig 2.15). Processed images recorded from each slice included: Raw, Cell, Volume, Brightness, Elongation, and Shape. Data generated from these images included: Slice Area (mm^2), Slice Brightness, Wall Thickness (mm), Non-Uniformity, Average Cell Elongation, Cell Angle to Vertical, Number of Cells, and Cell Diameter (mm). See Appendix A for descriptions of the parameters.

Figure 2.15 Bread Slicing for C-Cell Photos



Statistical Analysis

C-Cell data, baking data, and TLC flour lipid quantification were analyzed using ANOVA and Student's t-test on MS Excel software.

CHAPTER 3 - Results and Discussion

Results

Milling

Three samples of a commercially milled whole wheat flour (Table 3.1) were analyzed with a Ro-Tap Sieve Shaker to measure their cumulative granulation curves (Fig 3.1). From this data, an average curve was generated and used as the standard to match when designing a flow for milling the whole wheat flour in the KSU milling lab. A 95% confidence interval (Table 3.2) was generated from the WW commercial flour granulation data. Eight samples of HRW WW flour for milling practice were separately milled to whole wheat flour. Samples #1-5 were milled on the same day; samples 6-8 were all milled on a subsequent day. See Materials and Methods section for a complete description of the milling process. Duplicate samples from each milling were analyzed using the Ro-Tap, and the results compared to the average of WW Comm Ro-Tap results from previous testing (Table 3.3 and Fig 3.2).

Karl 92 WW flour was then milled so as to fall within the 95% confidence interval compared of the WW Comm flour granulation curve. See Table 3.4 for Karl 92 Ro-Tap Sieve Shaker data for the cumulative granulation curve (Fig 3.3). The objective was to produce a particle size distribution comparable to industrially milled whole wheat flours. Karl 92 flour fell within the 95% confidence interval of the WWComm Ro-Tap data (Table 3.2). Therefore, the lab scale milling flow produced whole wheat flours that were similar to commercially prepared flours with regard to their particle size distribution.

Table 3.1 Commercial WW flour Ro-Tap Sieve Shaker results for cumulative granulation curve

Over Sieve US Std #	Over (µm)	Cumulative %			
		WWCommA	WWCommB	WWCommC	WWComm Avg
20	841	0.00%	0.00%	0.10%	0.03%
30	594	1.81%	2.02%	2.01%	1.95%
40	420	6.34%	6.75%	6.93%	6.68%
60	250	13.98%	13.81%	14.47%	14.09%
100	150	31.89%	28.93%	36.78%	32.54%
PAN	0	100.00%	100.00%	100.00%	100.00%

Figure 3.1 Commercial WW cumulative granulation curves- three samples were drawn from the same lot of commercial whole wheat flour

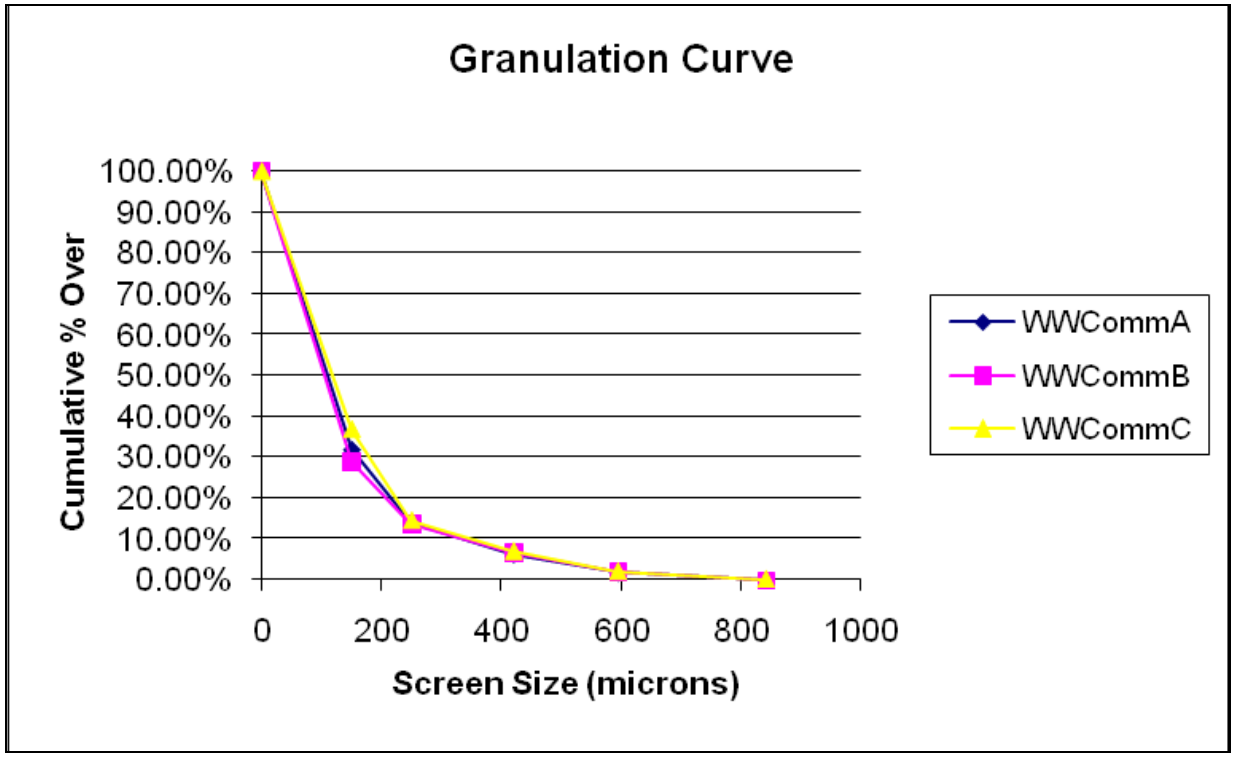


Table 3.2 A 95% Confidence interval for WW Comm flour from cumulative percent overs of Ro-Tap Sieve Shaker

Over Sieve US Std #	Over µm	WWComm Flour			
		AVG	STDEV	95% CI	
20	841	0.03%	0.06%	-3.25%	3.32%
30	594	1.95%	0.12%	-4.66%	8.55%
40	420	6.68%	0.31%	-10.64%	23.99%
60	250	14.09%	0.34%	-5.33%	33.51%
100	150	32.54%	3.97%	-191.84%	256.92%
PAN	0	100.00%	0.00%	100.00%	100.00%

Table 3.3 Granulation curve data for HRW WW flour for milling practice

Over Sieve	Over	Cumulative%																
US Std #	µm	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B	7A	7B	8A	8B	WWCommAVG
20	841	0.70%	0.40%	1.11%	1.31%	0.50%	0.60%	0.90%	1.01%	0.60%	0.60%	0.91%	0.81%	0.60%	0.60%	0.30%	0.30%	0.03%
30	594	1.61%	1.51%	2.72%	2.91%	1.61%	1.91%	2.21%	2.42%	1.51%	1.61%	2.01%	2.02%	1.81%	1.71%	1.01%	0.91%	1.95%
40	420	3.22%	3.32%	4.93%	5.23%	3.33%	3.71%	4.22%	4.54%	3.12%	3.23%	3.93%	3.83%	3.73%	3.63%	2.32%	2.22%	6.68%
60	250	11.36%	10.76%	12.29%	12.36%	10.49%	10.94%	11.36%	11.90%	9.97%	10.08%	11.08%	10.90%	10.89%	10.98%	9.67%	8.87%	14.09%
100	150	28.54%	25.86%	31.12%	28.54%	25.73%	25.30%	28.14%	28.23%	25.58%	25.40%	26.99%	26.34%	28.13%	28.00%	25.28%	23.19%	32.54%
Pan	0	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%

Figure 3.2 Cumulative granulation curve for replicate millings of a single HRW WW flour vs. the average of the commercial WW flour granulation curve

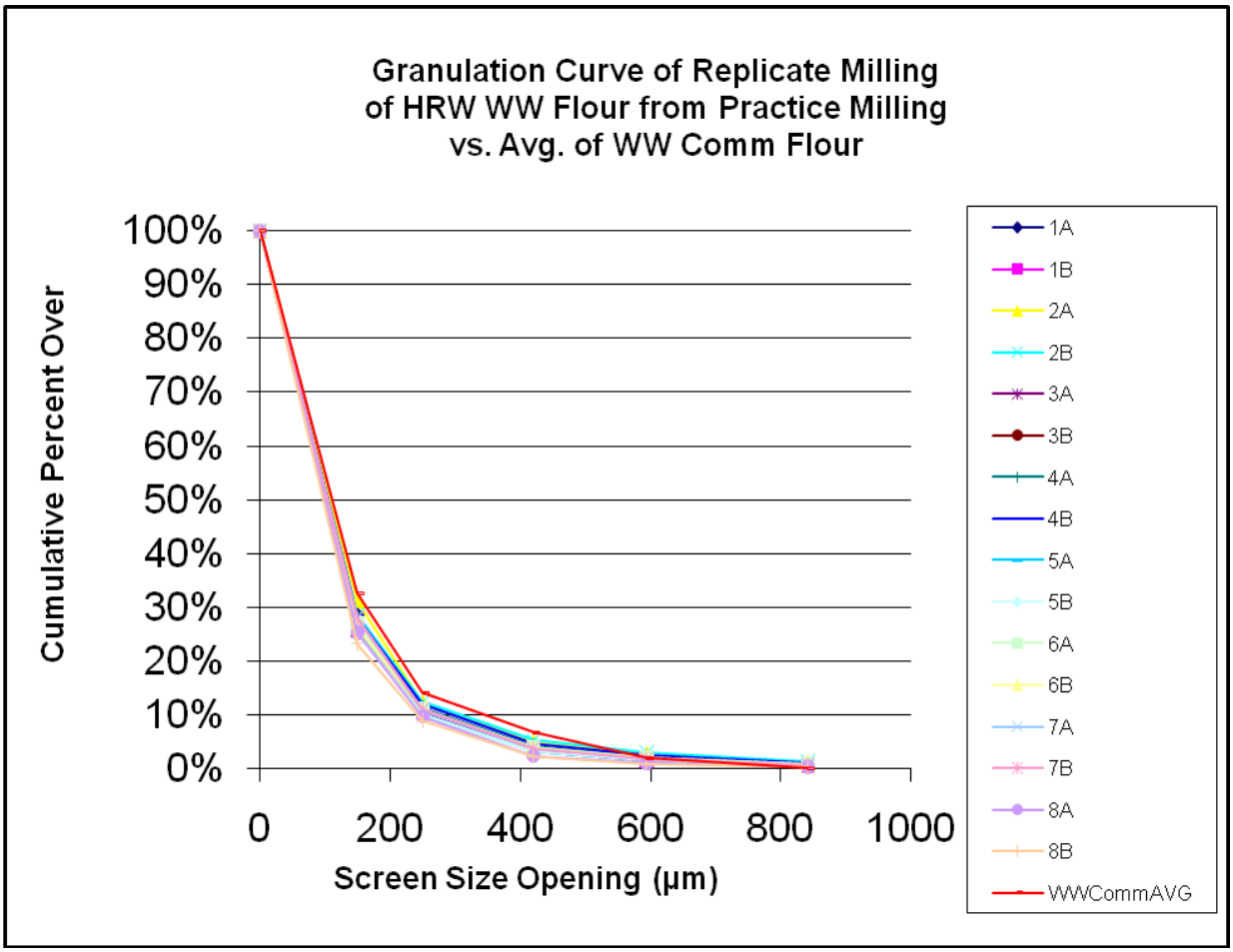
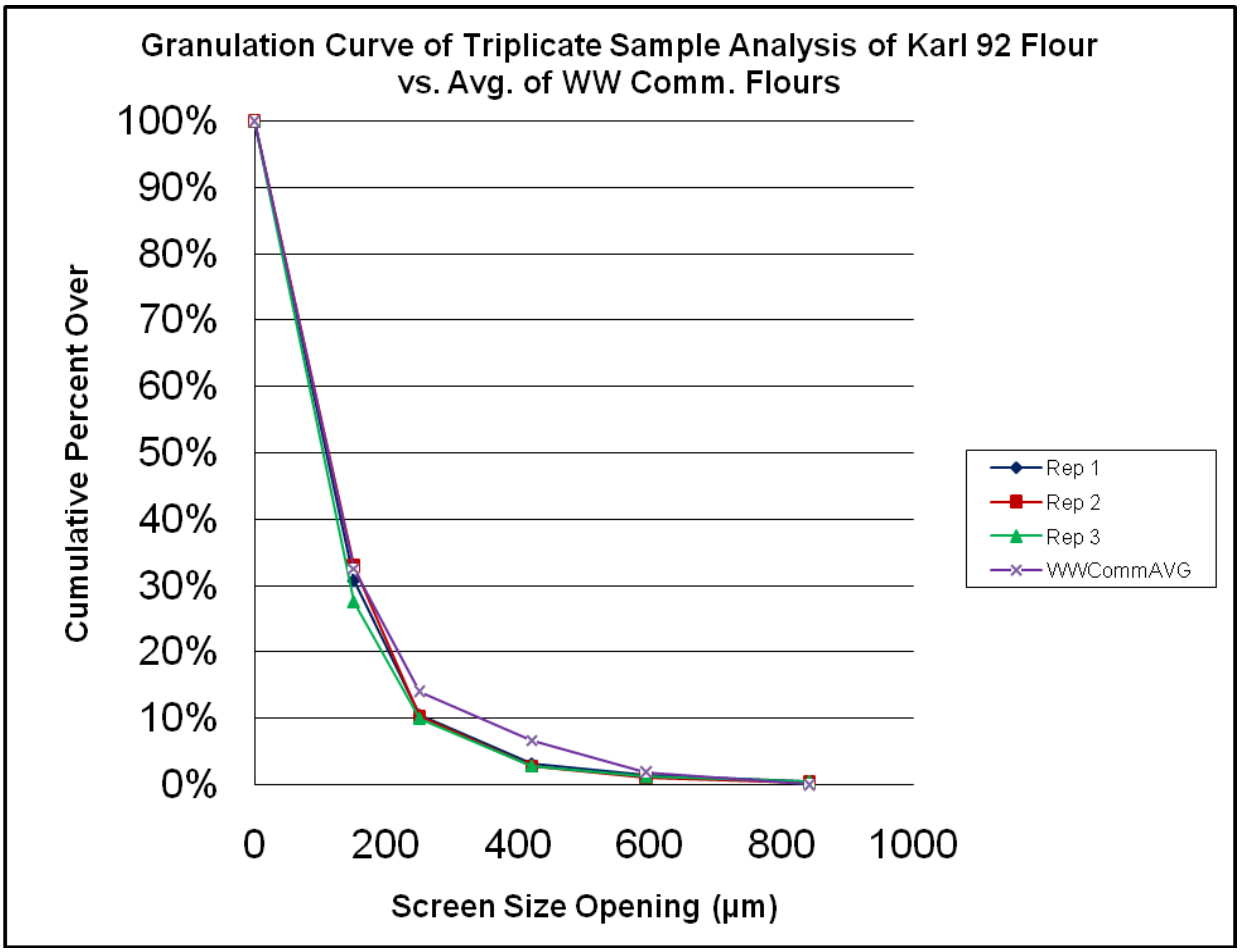


Table 3.4 Karl 92 WW flour Ro-Tap Sieve Shaker results for cumulative granulation curve

Over Sieve US Std #	Over (µm)	Cumulative % Overs				
		Karl 92 Rep 1	Karl 92 Rep 2	Karl 92 Rep 3	Karl 92 AVG	WWCommAVG
20	841	0.50%	0.40%	0.50%	0.47%	0.03%
30	594	1.41%	1.11%	1.21%	1.24%	1.95%
40	420	3.12%	2.83%	2.82%	2.92%	6.68%
60	250	10.47%	10.29%	9.98%	10.25%	14.09%
100	150	30.72%	33.00%	27.52%	30.41%	32.54%
Pan	0	100.00%	100.00%	100.00%	100.00%	100.00%

Figure 3.3 Cumulative granulation curve for duplicate samples from triplicate analysis of Karl 92 WW flour vs. the average of the commercial WW flour granulation curve



Falling Number

Commercial whole wheat flours are typically not malted at the mill, but need to be malted before baking. Therefore, all of the laboratory-milled flours were tested for their naturally occurring alpha-amylase level. The optimum level of malted barley flour (the desired source of alpha-amylase) to add to each flour in order to reach the desired range of 250-300 seconds was then determined (Table 3.5) using the Falling Number test.

Table 3.5 Falling Number of commercial and lab produced Karl 92 WW flours before and after malt added

	Unmalted FN (sec)		Malted FN
Sample ID	Rep 1	Rep 2	(sec)
KS 2[e]	671	663	223 [a]
KS 3[e]	714	748	275 [a]
KS 4[e]	720	710	264 [a]
KS 5[e]	600	604	223 [a]
KS 7[e]	739	739	256 [a]
Comm WW	487	485	243,244,238[b]
Polar Bear	N/A	N/A	225, 238 [c]
Karl 92	561	604	240,278[d]

- [a] 0.008 g malted barley flour per 7.00 g flour (1.82 oz malted barley flour/ cwt flour), (14% m.b.)
- [b] malted with 1.25% malted barley flour (20 oz malted barley flour/ cwt flour)
- [c] Polar Bear flour (a refined bread flour used as a control to standardize the pup loaf method) was commercially malted
- [d] 0.007 g malted barley flour per 7.00 g flour, (1.57 oz malted barley flour/ cwt flour), (14% m.b.)
- [e] for explanation of KS flours, see table 3.7
- typical commercial malt level is ~4 oz malted barley flour/ cwt flour (Pyler, 1988)

Dough Mixing

The moisture and protein content of Karl 92 WW flour were determined and used to estimate mixogram flour weight (as-is moisture basis) and to estimate mixogram absorption. The following calculations from the KSU Wheat Quality Lab were used to convert the flour moisture and protein content to estimate mixograph grams of flour, ml water, and absorption.

Mixograph calculations:

$\%\ Abs_{14} = (1.5 \times protein_{14}) + 43.6$

ml water = $[(Abs_{14})(0.1)] + (10 - g\ flour)$

g flour = $10 \times [(100-14)/(100 - mc\ flour)]$

$\%\ protein_{14} = [(100-14)/(100 - mc\ flour)\} \times orig\ protein\ \%$

Note: the tables in the Mixograph Handbook, which are based on the same calculation, could also be used to predict mixogram absorption.

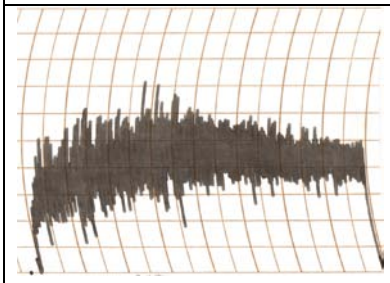
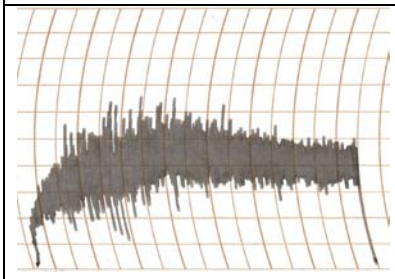
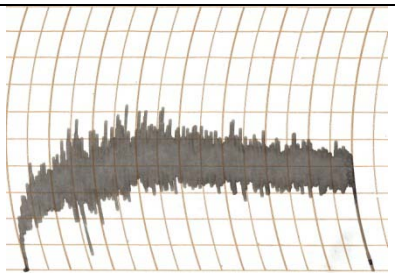
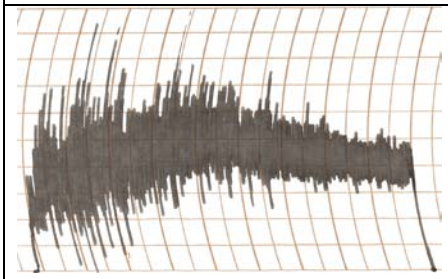
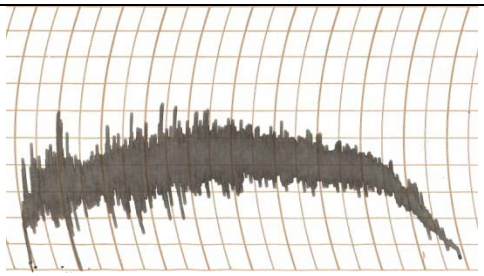
The calculated (predicted) absorption was used as the starting point in determining actual mixograph absorption by experiment. Test mixing and test baking at a range of water contents bracketing this absorption were used to further optimize absorption based on the scaled up, full-ingredient formula, the actual mixers used for baking, and assessment of dough handling properties during fermentation, sheeting, and proofing. Whole wheat flours absorbed more water throughout the baking process than did white flours, thus requiring more water in formula. The following formulas were, thus, developed and determined to be a simple, accurate calculation to standardize absorption within flour type (refined/white, or whole wheat):

- ml water used in tall (50 mL) burette for white flours = optimized flour-water mixograph absorption minus 20
- ml water used in tall (50 mL) burette for whole wheat flours = optimized flour-water mixograph absorption minus 15

Mixograms

Figure 3.6 shows the mixograms obtained at the predicted and optimized baking absorption for each of the flours. The White Flour Control and Whole Wheat Commercial Control were used earlier in the experiment to standardize the baking method. The Hudson Cream, King Arthur, and KSU roller mill flours were the aged whole wheat flours used to gather preliminary data on whole wheat flour general mixing and baking characteristics. The Karl 92 WW flour was laboratory milled and used in subsequent experiments. Table 3.7 lists the mixing times for predicted and optimized absorptions of the Mixographs from Table 3.6.

Table 3.6 Mixograms of control, aged WW, and Karl 92 flours

Predicted Absorptions	Optimized Absorptions
 PB White Ctrl: 9.89 g, 6.1 mL	PB White Ctrl: actual is same as predicted absorption
 WW Comm Ctrl: 9.79 g, 6.6 mL	 WW Comm Ctrl: 9.79 g, 6.7 mL
 Hudson Cream 100% Hard White WW Flour: 9.40 g, 6.9 mL	 Hudson Cream 100% Hard White WW Flour: 9.40 g, 7.7 mL

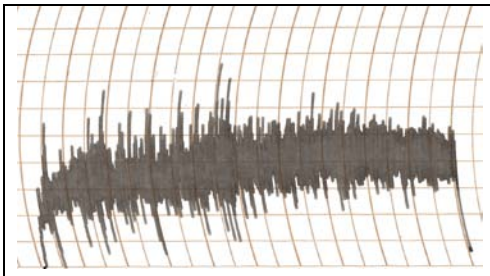
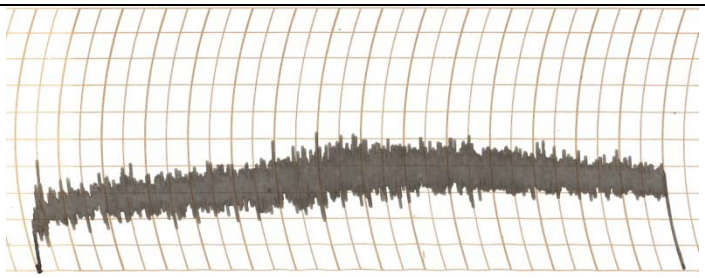
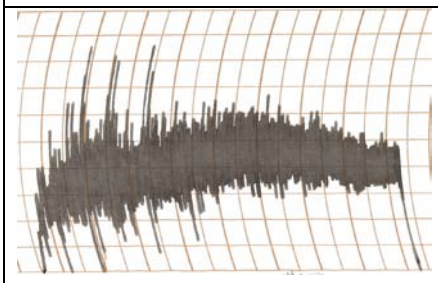
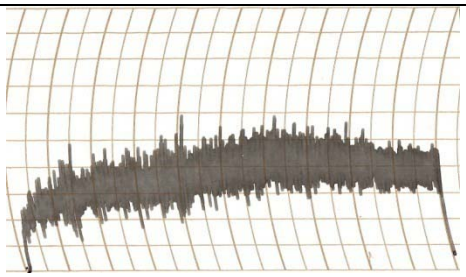
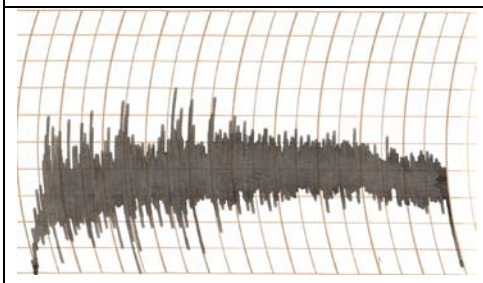
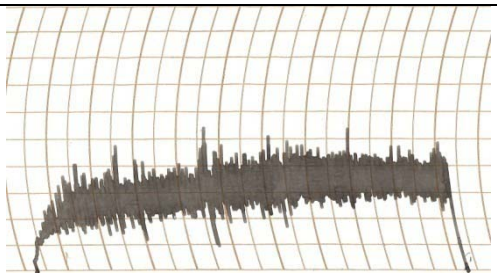
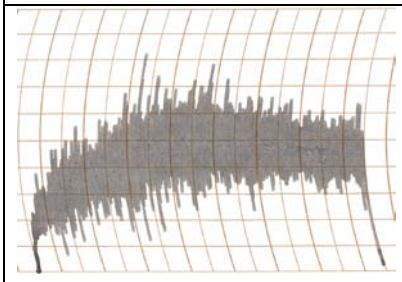
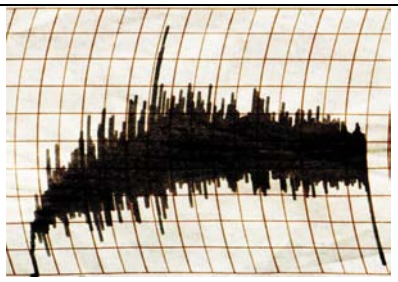
 King Arthur All-Natural Hard White WW Flour: 9.42 g, 6.85 mL	 King Arthur All-Natural Hard White WW Flour: 9.42 g, 7.45 mL
 King Arthur All-Natural Traditional Hard Red WW Flour: 9.42 g, 7.05 mL	 King Arthur All-Natural Traditional Hard Red WW Flour: 9.42 g, 7.6 mL
 WW (KSU roller mill), not tempered: 9.60 g, 6.55 mL	 WW (KSU roller mill), not tempered: 9.60 g, 7.15 mL
 Karl 92: 9.81 g, 6.75 mL	 Karl 92: 9.81 g, 6.95 mL

Table 3.7 Mixing time at predicted and optimized Mixograph absorption. (See Table 3.8 for predicted and optimized absorption levels.)

Sample ID	Sample Type	Mix Time at Predicted Mixograph Absorption (min:sec)	Mix Time at Mixograph Optimized Absorption (min:sec)
Polar Bear	ADM Polar Bear commercial refined flour	3:30	3:15
Comm WW	Commercial whole wheat flour (not aged)	3:45	3:15
KS 2	Hudson Cream 100% WW Flour	4:15	4:30
KS 3	King Arthur All-Natural White WW Flour	7:00	7:45
KS 4	King Arthur All-Natural (Red) Traditional WW Flour	5:15	6:15
KS 7	Whole Wheat (KSU roller mill), not tempered	5:30	6:45
Karl 92	Karl 92 Lab-Milled HRW WW for 21-day Baking	4:30 at 6.75 mL, 4:45-6:00 at 7.15 mL	4:00

The white flour control was a strong bread baking flour. The whole wheat control was a strong whole wheat flour intended for bread baking. Most of the aged whole wheat flours had very long mixing times. While the reason for this is unknown, it may be due to the additional oxidative change that took place over the storage time. These same flours also had high mixing tolerance, and no clear point at which they broke down, which is typical of WW flours. The high absorption (see Table 3.8) was necessary with the aged whole wheat flours in order to achieve optimum handling properties. Although the Polar Bear flour was aged, it did not require a high absorption. As a refined (not whole wheat) flour it contained much less bran, which generally increases absorption.

Table 3.8 Protein and moisture content, Mixograph optimization, and test mixing and baking results for various flours

Sample ID	Sample Type	Total N %	Protein % as-is	Protein % 14 mb	Moisture %	Mixograph Flour (g)	Mixograph Predicted (ml water)	Mixograph Optimum (ml water)	Baking Actual (ml Water)	Mix Time Actual (min)
KS 2	Hudson Cream 100% Whole Wheat Flour	2.40	13.68	12.86	8.54	9.40	6.89	7.70	88.7	6:30
KS 3	King Arthur All-Natural White Whole Wheat Flour	2.37	13.51	12.72	8.70	9.42	6.85	7.45	86.3	9:45
KS 4	King Arthur All-Natural Traditional Whole Wheat Flour	2.58	14.71	13.87	8.81	9.43	7.01	7.60	87.7	7:30
KS 7	Whole Wheat (KSU roller mill), not tempered	2.16	12.31	11.82	10.39	9.60	6.54	7.15	83.3	7:45
Polar Bear	ADM Polar Bear commercial refined flour	1.91	10.91	10.80	13.10	9.89	6.09	6.10	67.3	3:40
Comm WW	Commercial whole wheat flour (not aged)	2.43	13.87	13.58	12.18	9.79	6.61	6.70	78.3	5:45
Karl 92	Karl 92 Hard Red Whole Wheat for 21-day Baking	2.72	15.49	15.20	12.37	9.81	6.83	6.95	81.7	8:45

As can be seen from Table 3.8, flour mixing and baking properties varied based on different protein quantity (as well as quality), wheat type, flour type (WW vs. white/refined), presence or absence of tempering (Karl 92 WW flour was also untempered), and duration of storage.

Preliminary Bake Testing

Four WW flours (Table 3.8) which had been aged at room temperature for several months were analyzed with the Mixograph to optimize absorption and baked along with the control refined flour and control WW flour, using Method 10-10b (AACCI, 2000), as described in methods section, in order to generate preliminary results as a guide to final method development.

Baking Results

The optimized formula (below) was the result of test baking optimization with white control flour and whole wheat commercial control flour (Table 3.8). The formula follows

Method 10-10b (AACCI, 2000) with the following exceptions: yeast level was optimized based on proof height obtained at recommended proof time, alpha-amylase level was determined based on individual flours. The method and its details are presented in detail in Materials and Methods. Several aged WW flours were baked at the same time, at an amylase supplementation level to put all flours within the desired Falling Number range. The absorption level was optimized for each flour.

Optimized Baking Formula:

Flour (14% mb)	100%
Shortening	3%
Salt[a]	1.5%
Sucrose[a]	6%
Yeast (active dry)	2.0%
Alpha amylase[b]	0.098%
Ascorbic Acid	50 ppm; 5 ml/100 g flour
Water	optimum (see calculations in Mixograms section)

[a] added in aqueous solution

[b] malted barley flour

All ingredients are on a flour weight basis.

Variation between formulas using different flours was in absorption level only.

In order to test if low temp could halt any time related changes occurring in WW flour baking quality, laboratory milled flours were stored under two conditions (at room temperature and in a -15°F freezer), as this was found to be the case for soft wheats and soft wheat flour (Shelke et al., 1992a). Baking quality was assessed using bread specific volume, bread loaf volume, bread loaf weight, and dough proof height, as well as the C-Cell properties of slice area, slice brightness, number of cells, wall thickness, cell diameter, non-uniformity, average cell elongation, and cell angle to vertical. All results were the means of three replications.

Proof height, loaf weight, and loaf volume were measured for Karl 92 flours stored at RT and Frzr temp (Tables 3.9 and 3.10).

Table 3.9 Baking properties of Karl 92 WW flour storage at Frzr temp during 21-day storage study

	Freezer Temp		
	MEAN		
Day	Proof Ht. (cm)	Loaf Wt. (g)	Loaf Vol. (cc)
0	7.58 ± 0.23	163.34 ± 2.25	666.67 ± 24.25
3	7.48 ± 0.21	163.97 ± 0.90	656.67 ± 29.78
6	7.50 ± 0.19	162.57 ± 1.93	654.17 ± 33.08
9	7.28 ± 0.15	163.30 ± 2.42	599.17 ± 51.52
12	7.48 ± 0.13	164.02 ± 1.04	671.00 ± 51.28
15	7.66 ± 0.26	163.74 ± 0.76	682.00 ± 23.35
18	7.58 ± 0.24	163.76 ± 0.29	672.50 ± 21.85
21	7.52 ± 0.16	164.87 ± 2.58	665.00 ± 26.08

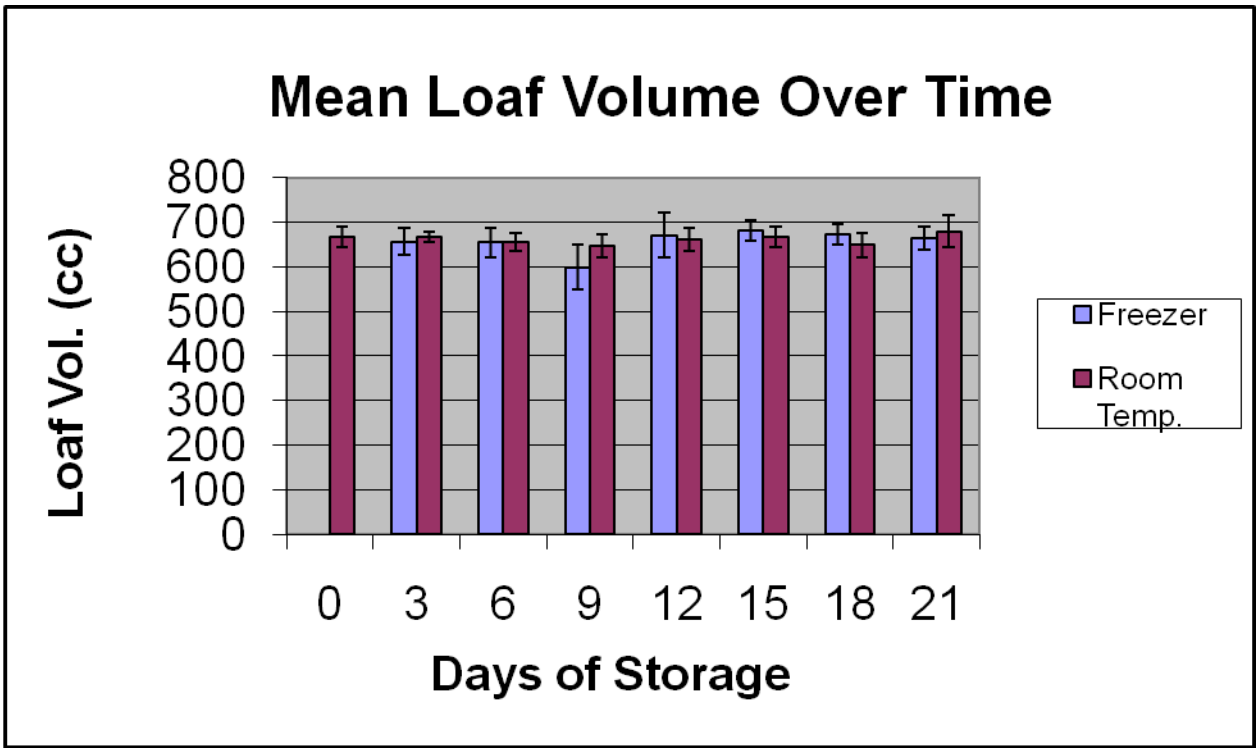
Table 3.10 Baking properties of Karl 92 WW flour stored at RT during 21-day storage study

	Room Temp				
	MEAN			Percent St. Dev.	
Day	Proof Ht. (cm)	Loaf Wt. (g)	Loaf Vol. (cc)	Proof Ht.	Loaf Vol.
0	7.58 ± 0.23	163.34 ± 2.25	666.67 ± 24.25	3.03%	3.64%
3	7.60 ± 0.15	162.61 ± 1.65	667.50 ± 12.14	1.97%	1.82%
6	7.72 ± 0.26	163.90 ± 1.81	655.00 ± 20.98	3.37%	3.20%
9	7.47 ± 0.21	164.07 ± 1.32	646.67 ± 24.63	2.81%	3.81%
12	7.60 ± 0.11	162.84 ± 1.75	661.67 ± 25.63	1.45%	3.87%
15	7.60 ± 0.22	164.29 ± 0.99	666.67 ± 24.22	2.89%	3.63%
18	7.44 ± 0.26	163.91 ± 1.66	648.33 ± 26.96	3.49%	4.16%
21	7.72 ± 0.31	164.02 ± 2.62	679.17 ± 35.56	4.02%	5.24%

Standard deviations for proof height and loaf volume were acceptable, ranging from a low of 1.45% and 1.82% for proof height and loaf volume respectively to a high of 4.02% and 5.24% (Table 3.10).

The data is graphically displayed in Fig 3.4 to show trends between freezer and room temperature flour when baked. (Day 0 had only one temperature of sample, since no time had elapsed for storage at either temperature.)

Figure 3.4 Loaf volume mean and standard deviation for the 21-day baking study of Karl 92 WW flour stored at room temperature and in freezer



The results (Table 3.9 and 3.10) showed no clear trend in loaf volume for flours stored under either condition. Data analysis (Table 3.10) indicated standard deviations for loaf volume values over storage time overlapped each other. ANOVA was used to pinpoint significant differences in baking characteristics and final bread characteristics as measured by C-Cell (5% confidence level). However, there were no consistent trends for either storage temp or storage time.

Karl 92, the wheat used as the basis for this study is a very strong, high protein variety. It may be that its strong breadmaking characteristics obscured or buffered any post-milling deteriorations in quality that would have been manifested using a weaker wheat.

Baking and C-Cell Results: ANOVA

Data was analyzed by a 2-Factor ANOVA with Replications using MS Excel and evaluated by comparing F-value vs. F-critical value. Results are shown below in Tables 3.11 through 3.18. Some of the parameters showed significant differences as indicated by an F-value greater than F-critical value. Those parameters are discussed below.

Statistically Significant Results

The ANOVA for bread volume, slice area, slice brightness, wall thickness, cell diameter, and non-uniformity showed significant differences for storage time. Thus, at least one of the eight baking days produced values statistically significantly different from that of the other days for each of these parameters.

Table 3.11 Bread Loaf Volume

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Days	22139.91	7	3162.844	3.607592	0.001992	2.126324
Storage Temp	119.2604	1	119.2604	0.13603	0.713234	3.960352
Interaction	10457.82	7	1493.975	1.704052	0.119816	2.126324
Within	70137.5	80	876.7188			
Total	102854.5	95				

Average bread loaf volume was significant for Days, but not Storage Temperature (Table 3.11).

Table 3.12 Bread Specific Volume

ANOVA 5%

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Days	0.802096	7	0.114585	3.436607	0.002896	2.126324
Storage Temp	0.002469	1	0.002469	0.074059	0.786217	3.960352
Interaction	0.415008	7	0.059287	1.778115	0.103139	2.126324
Within (Error)	2.667401	80	0.033343			
Total	3.886973	95				

The average specific volumes and the average volumes of Room Temp (RT) samples were greater than those of the Freezer (Frzr) samples (Table 3.12). However, the values were significant only for Days, not for Temperature. Because these two variables are related by a calculation, this is to be expected.

An important point to note is that there was no significant trend in loaf volume as a function of either storage time or storage temperature. The original hypothesis was that time would affect baking quality as reflected by changes in loaf volume and that putting the flour at low temp would stop or slow those changes. Possible explanations for the lack of differences are listed on page 82-83 and in the Conclusions.

Table 3.13 Slice Area (mm^2)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	883625.5	7	126232.2	3.094068	0.006138	2.126324
Storage Temp	22411.48	1	22411.48	0.549326	0.460763	3.960352
Interaction	419189.9	7	59884.27	1.467819	0.190693	2.126324
Within	3263850	80	40798.13			
Total	4589077	95				

Average slice area of RT Samples was also greater than Frzr Samples, but only significant for Days, not for Temperature (Table 3.13). Therefore, any changes due to flour age were not affected by differences in storage temperatures.

Table 3.14 Wall Thickness (mm)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	0.001268	7	0.000181	3.510492	0.002463	2.126324
Storage Temp	9.09E-05	1	9.09E-05	1.761467	0.188217	3.960352
Interaction	0.000424	7	6.06E-05	1.175136	0.326425	2.126324
Within	0.004127	80	5.16E-05			
Total	0.00591	95				

Wall thickness was significantly greater across Days for Frzr Samples than for RT Samples (Table 3.14). This is explained by the specific volume results. The Frzr Samples were denser, consequently the loaves had more material in a smaller space, and thus the cell walls were thicker.

Table 3.15 Slice Brightness

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Days	107.2508	7	15.32154	4.995307	9.99E-05	2.126324
Storage Temp	8.366204	1	8.366204	2.727647	0.102545	3.960352
Interaction	26.5523	7	3.793185	1.236698	0.292809	2.126324
Within	245.375	80	3.067188			
Total	387.5443	95				

Average brightness of RT Samples was greater than Frzr Samples, but only significant for Days (Table 3.15). This may be related to differences in cell size, which would affect sample reflectivity. Smaller cells would result in increased brightness.

Table 3.16 Cell Diameter (mm)

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Days	0.123812	7	0.017687	3.156179	0.005357	2.126324
Storage Temp	0.038432	1	0.038432	6.857876	0.010553	3.960352
Interaction	0.020488	7	0.002927	0.522268	0.815251	2.126324
Within	0.448325	80	0.005604			
Total	0.631057	95				

Cell diameter was significantly greater for Frzr Samples, both across days and across temperatures (Table 3.16). It could be that the flour aging process occurring at room temperature promoted a gluten network with finer/smaller cells.

Table 3.17 Non-Uniformity

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Days	105.9612	7	15.13732	2.664647	0.015699	2.126324
Storage Temp	27.8103	1	27.8103	4.895491	0.029778	3.960352
Interaction	115.0146	7	16.43065	2.892314	0.00955	2.126324
Within	454.4639	80	5.680799			
Total	703.25	95				

Non-uniformity was greater for RT Samples than for Frzr Samples; and was significant across Days, Temperature, and the interaction between Days and Temperature (Table 3.17). Despite the fact that the RT samples had a finer cell structure, it appears that the cells did not all expand evenly. This is normal, because different sections of a bread slice normally have different cell sizes and shapes. However, because this is an average value for all the cells measured on the slice, it may not hold any real meaning in this case. This is a limitation of the

C-Cell data in that it measures cell diameters with the assumption that all cells in the bread would normally be similarly sized and reports an average value over the entire slice.

Statistically Insignificant Results

Parameters that did not have statistically significant differences with storage time included: bread loaf weight, dough proof height, number of cells, average cell elongation, and cell angle to vertical. These parameters may have responded to better experimental control, or are simply not correlated with storage conditions or age of flour.

Table 3.18 Bread Loaf Weight

ANOVA

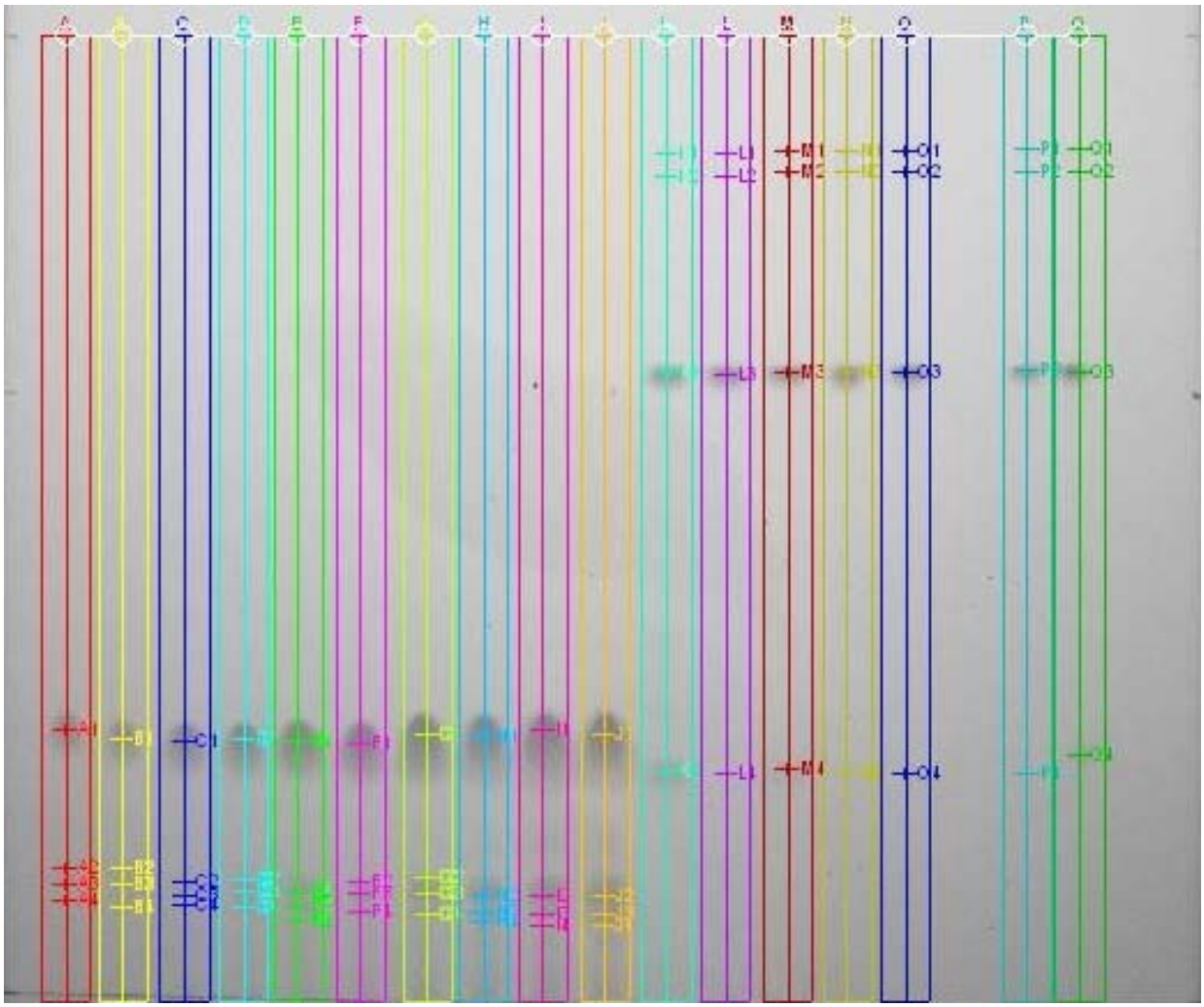
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	14.92957	7	2.132796	0.690372	0.679865	2.126324
Storage Temp	0.416594	1	0.416594	0.134849	0.714427	3.960352
Interaction	27.57942	7	3.939917	1.275325	0.27315	2.126324
Within	247.1474	80	3.089343			
Total	290.073	95				

The fact that average bread loaf weight was not significant over Days or Storage Temp (Table 3.18) indicates good experimenter consistency in measuring ingredients, and handling the dough ball throughout proofing/fermentation and sheeting/molding process. If those factors were not well-controlled, there could be differences in loaf weight if samples proofed or baked very differently from each other. That was not seen in these experiments.

Thin Layer Chromatography

The thin layer chromatography results were analyzed quantitatively by scanning the developed plates and subjecting the results to the Doc-It software program for quantification (Fig 3.5). The results were analyzed by ANOVA, Two Factor with Replication.

Figure 3.5 Sample TLC plate analyzed with Doc-It software



Figures 3.6 through 3.10 present the cumulative intensity vs. storage time of the three flour glycolipid fractions (free, bound, and total) as a function of storage time. Separate plots on each figure show changes during frozen vs. room temperature flour storage.

Tables 3.19 through 3.21 are the ANOVAs that correspond to free, bound, and total glycolipids, respectively.

Figure 3.6 Glyco-Free by storage condition over time

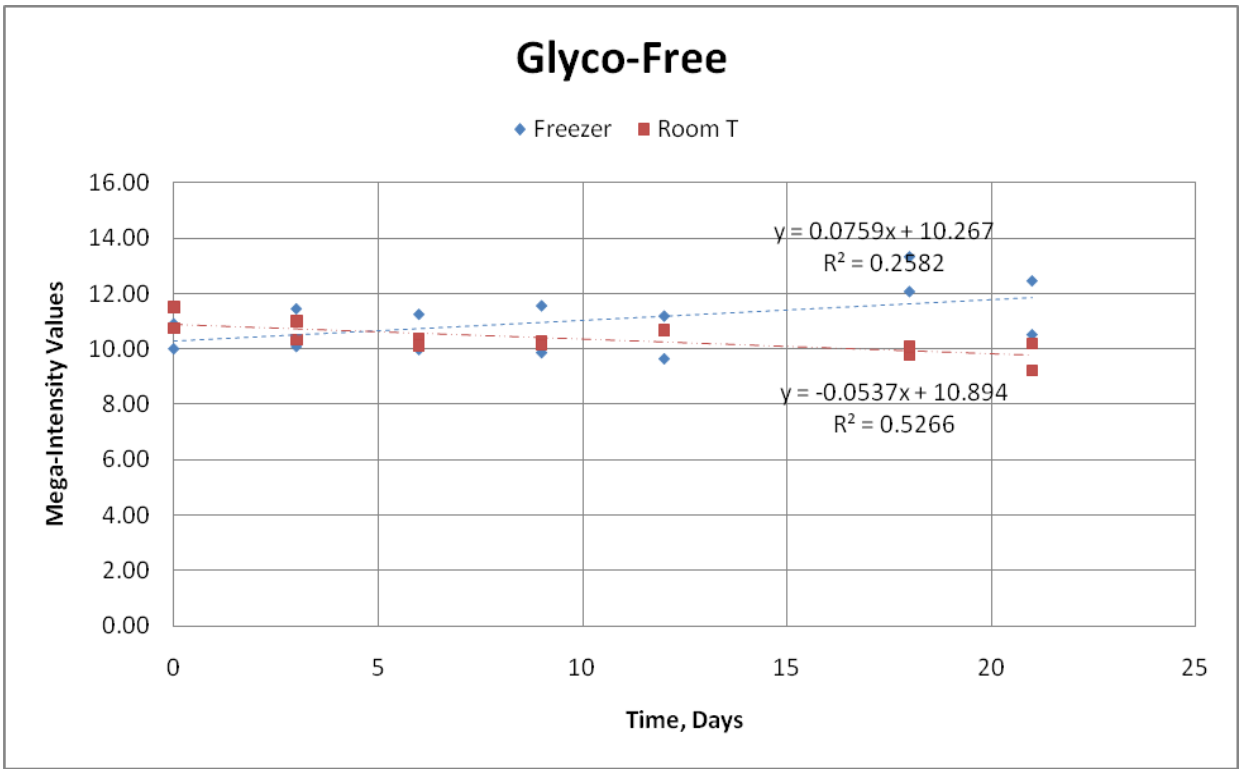


Figure 3.7 Glyco-Bound by storage condition over time

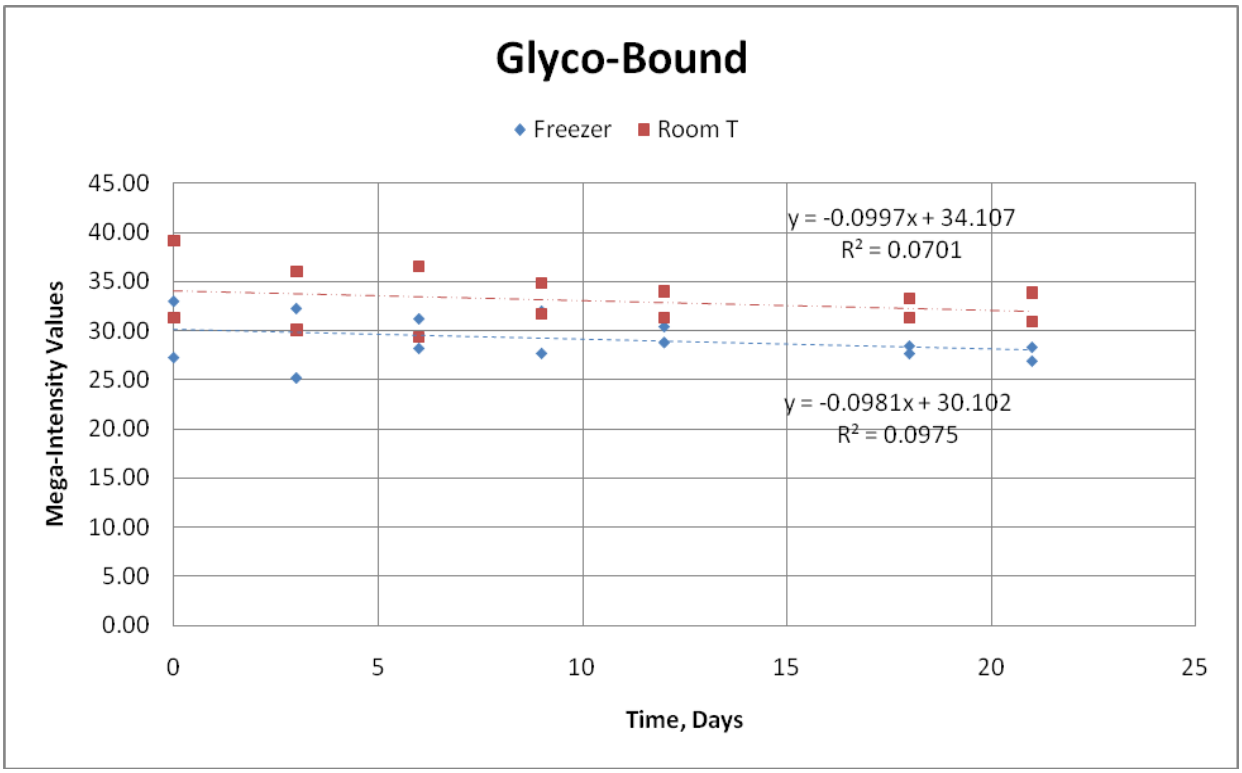


Figure 3.8 Glyco-Total by storage condition over time

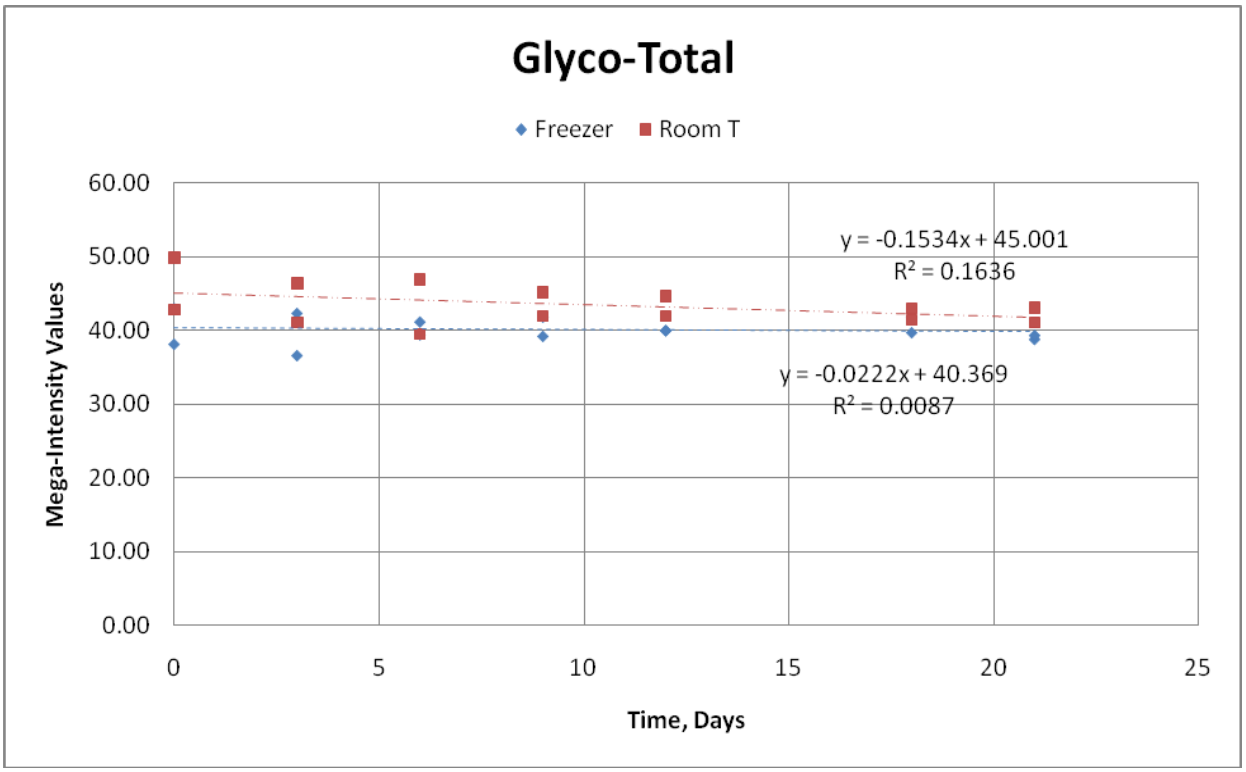


Figure 3.9 Glyco-Frzr by free, bound, total, over time

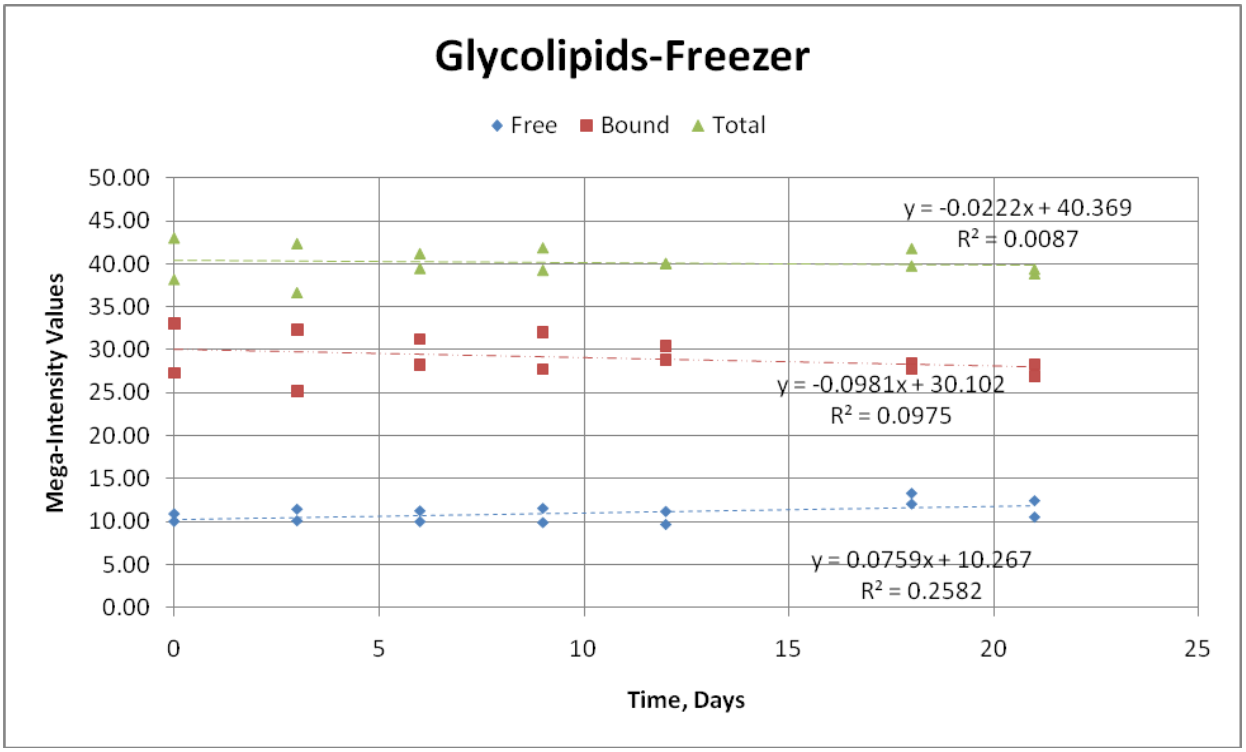


Figure 3.10 Glyco-RT by free, bound, total, over time

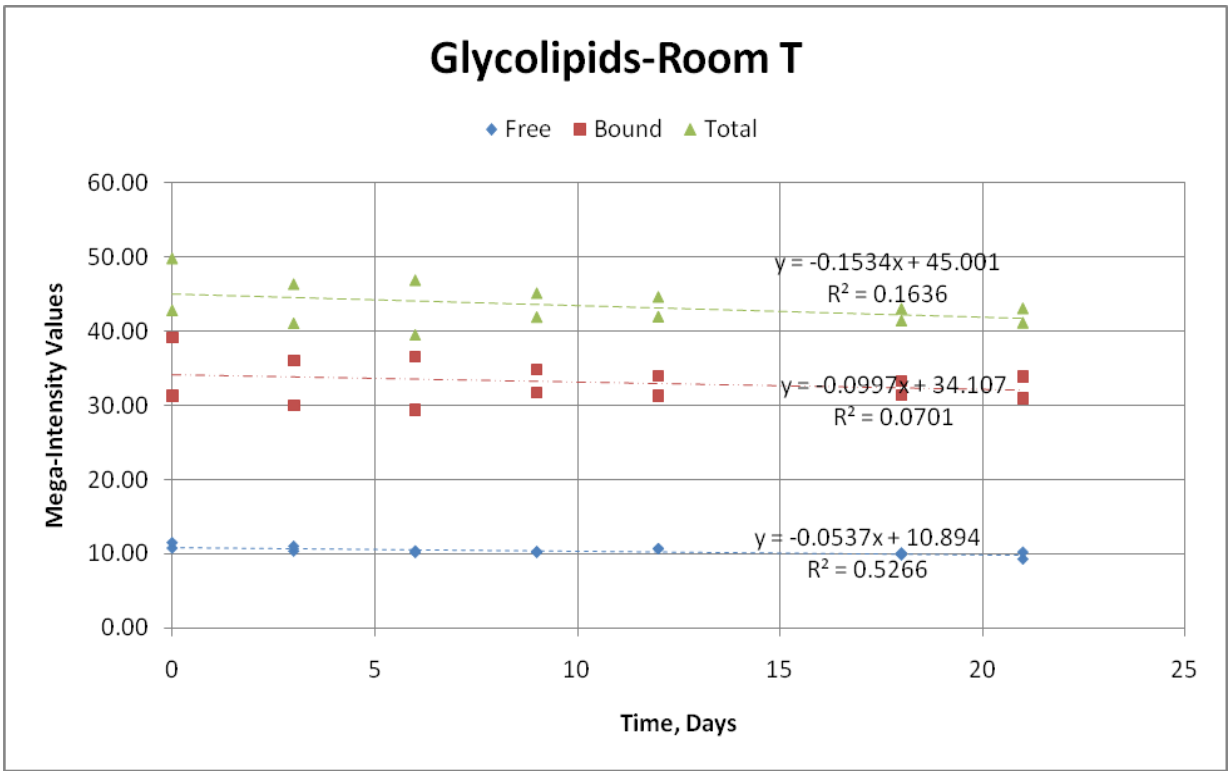


Table 3.19 Glyco-Free ANOVA

	Total	*Freezer*	*Room*
Count		14	14
Sum		154.2148	145.0942
Average		11.01534	10.36387
Variance		1.217097	0.299329

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	2.280649	6	0.380108	0.615205	0.715115	2.847726
Storage Temperature	2.97089	1	2.97089	4.808386	0.045713	4.60011
Interaction	8.782907	6	1.463818	2.36919	0.086148	2.847726
Within	8.649982	14	0.617856			
Total	22.68443	27				

Table 3.20 Glyco-Bound ANOVA

	Total	*Freezer*	*Room*
Count		14	14
Sum		407.8932	463.7409
Average		29.13523	33.12435
Variance		5.382153	7.738583

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	19.48422	6	3.24737	0.308588	0.922041	2.847726
Storage Temperature	111.3916	1	111.3916	10.58522	0.005772	4.60011
Interaction	3.758855	6	0.626476	0.059532	0.998844	2.847726
Within	147.3265	14	10.52332			
Total	281.9612	27				

Table 3.21 Glyco-Total ANOVA

	Total	*Freezer*	*Room*
Count		14	14
Sum		562.108	608.8351
Average		40.15057	43.48822
Variance		3.080649	7.855829

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	17.6321	6	2.938683	0.360798	0.891715	2.847726
Storage Temperature	77.97944	1	77.97944	9.573951	0.007923	4.60011
Interaction	10.51269	6	1.752115	0.215116	0.965717	2.847726
Within	114.0294	14	8.144959			
Total	220.1537	27				

Neither storage time nor storage temperature had a significant effect on the amount of free glycolipids as detected by TLC (see Table 3.19). There did appear to be a slight tendency for the free glycolipids (FGL) to increase during freezer storage, and for the Room Temperature

FGL to decrease during that time (Fig 3.6). However, the Day-18 data for Frzr storage flour was inconsistent with the rest of the data. If Day-18 data is eliminated from the data set, there is a large drop in both storage F-value and correlation coefficient for Frzr storage such that they are no longer significant. No experimental explanation was apparent for Day 18 data. Bound glycolipids (BGL) did not appear to be affected by storage time or temperature, although RT samples were consistently higher in intensity than Frzr samples (Table 3.20). Both storage conditions resulted in a slight decrease in intensity over time. Total Glycolipids (TGL) were significant for storage temp, but not for days (Table 3.21). RT exhibited a slight decrease over storage time, while Frzr samples remained roughly constant (Fig 3.8). When evaluating glycolipids by storage temperature, a slight decrease across storage time was apparent for both Frzr and RT Glycolipids at each of the total, free, and bound levels (Fig 3.9 and 3.10).

Figures 3.11 through 3.15 present the cumulative intensity in storage time of the three flour phospholipid fractions (free, bound, and total) as a function of storage time. Separate slots on each figure show changes during frozen vs. room temperature flour storage.

Tables 3.22 through 3.24 are the ANOVAs that correspond to free, bound, and total phospholipids, respectively.

Figure 3.11 Phospho-Free by storage condition over time

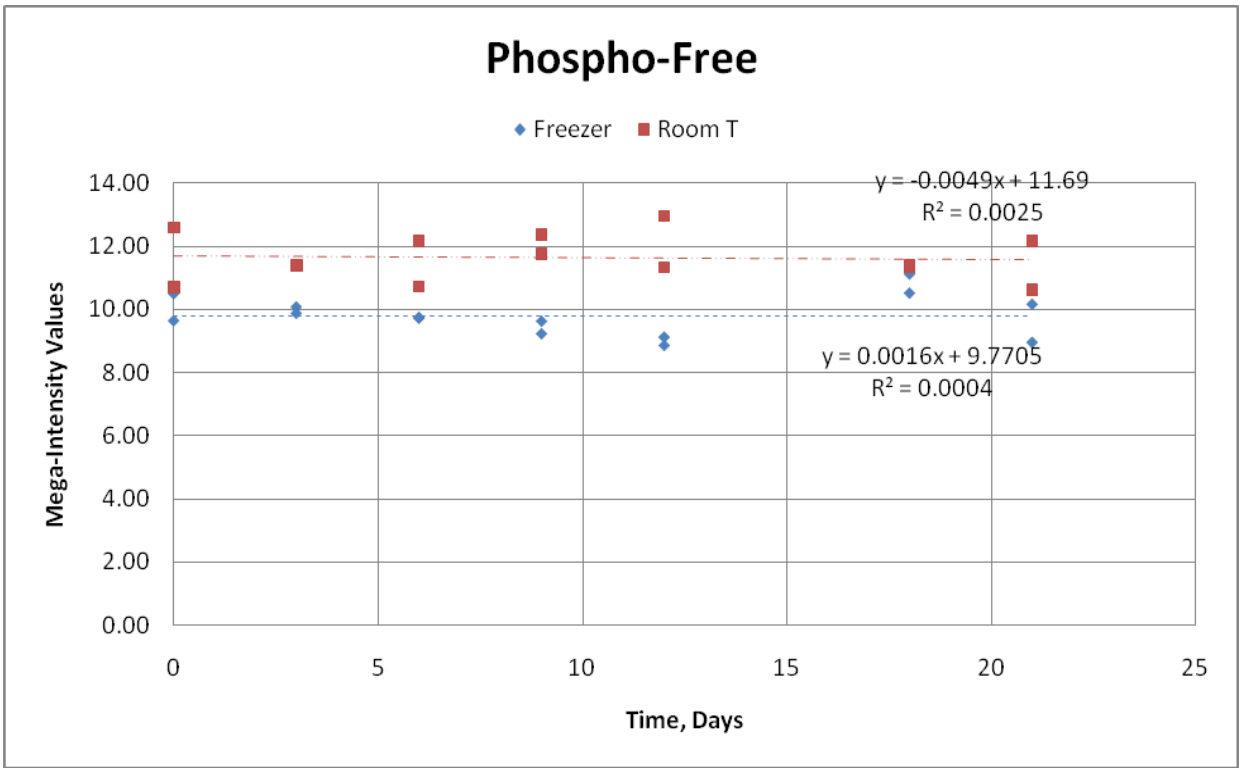


Figure 3.12 Phospho-Bound by storage condition over time

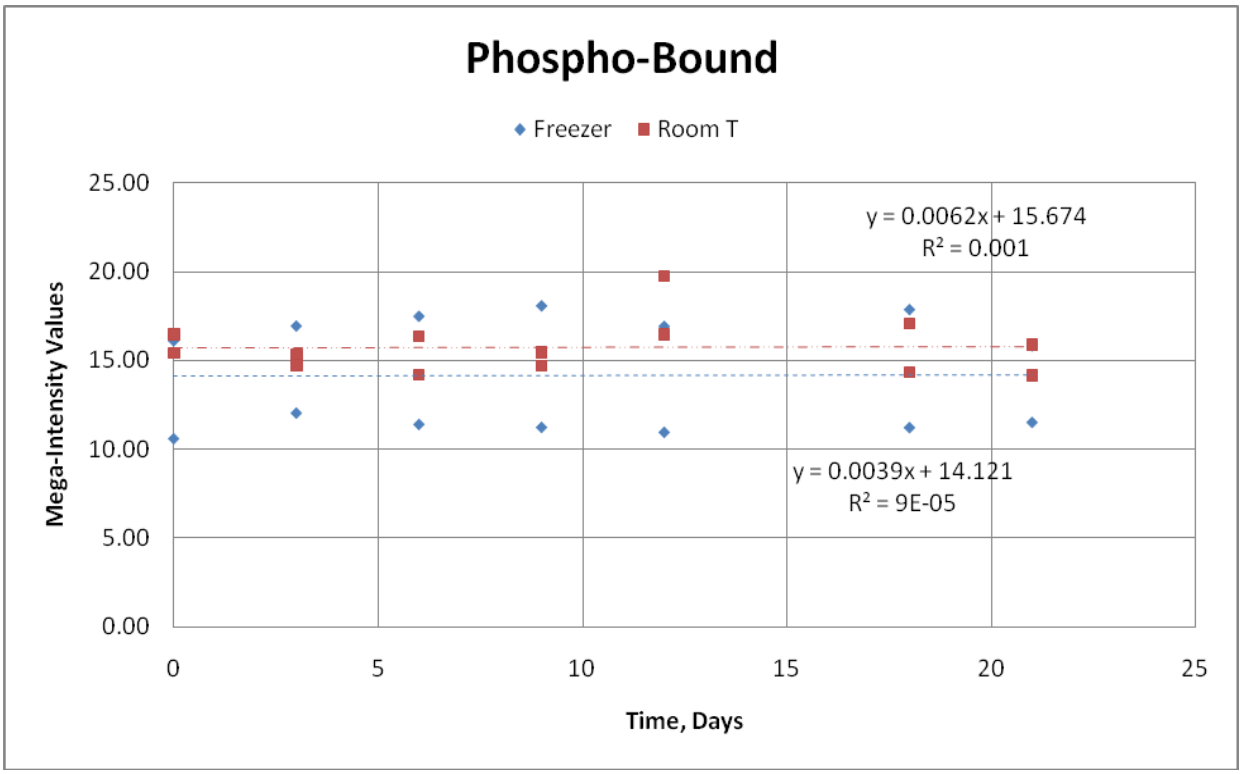


Figure 3.13 Phospho-Total by storage condition over time

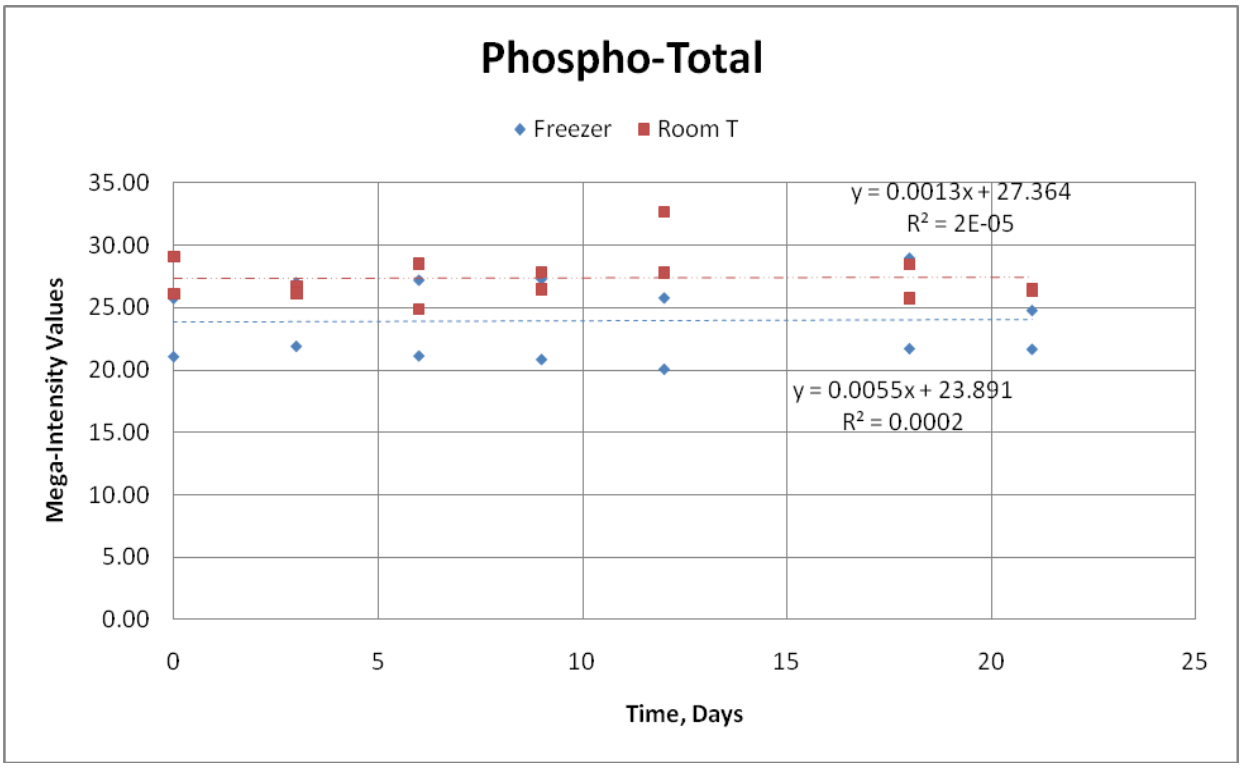


Figure 3.14 Phospho-Frzr by free, bound, total, over time

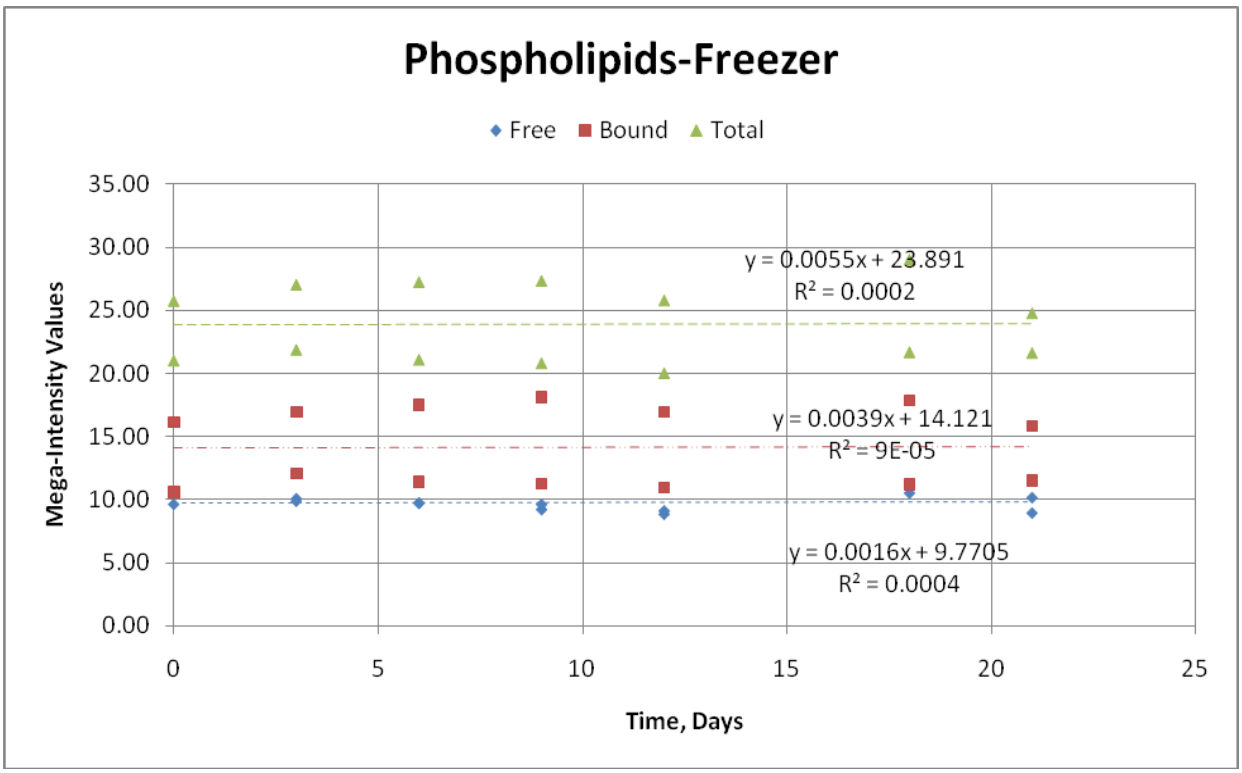


Figure 3.15 Phospho-RT by free, bound, total, over time

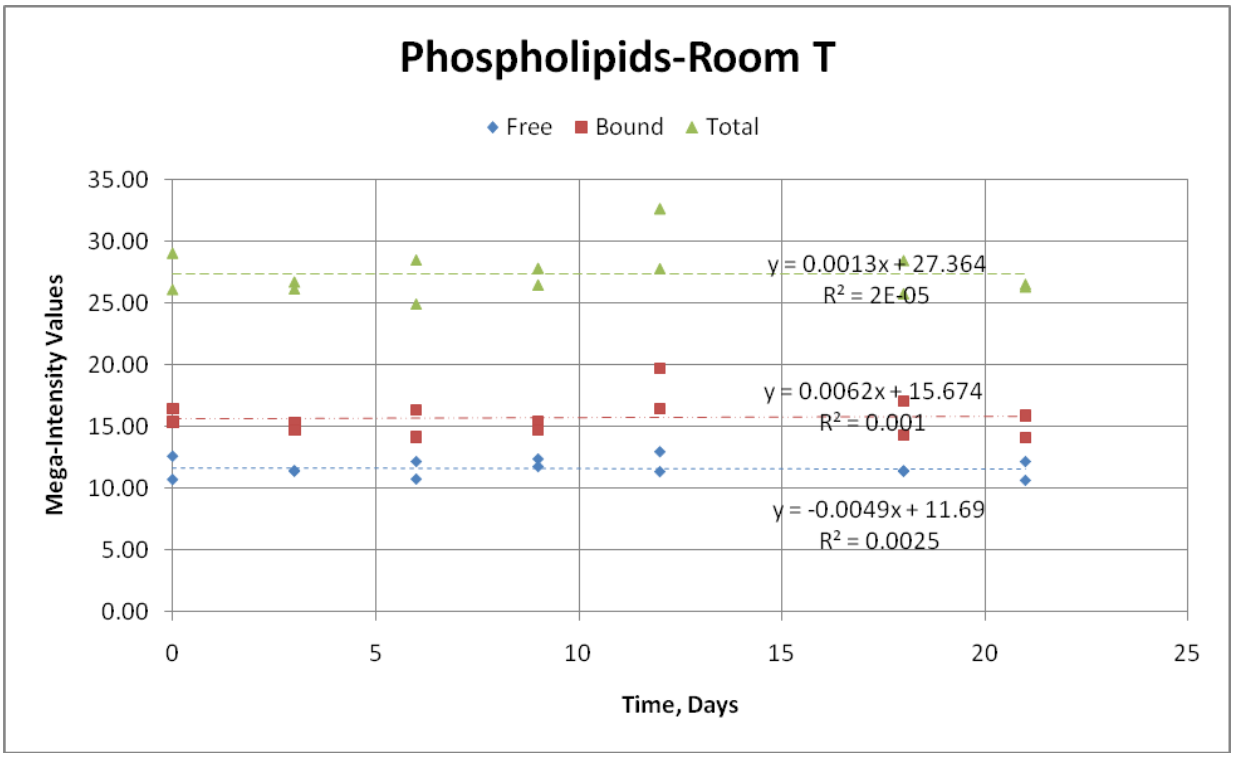


Table 3.22 Phospho-Free ANOVA

	Total	*Freezer*	*Room*
Count		14	14
Sum		137.0143	162.9843
Average		9.786733	11.64174
Variance		0.412064	0.527688

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	1.054682	6	0.17578	0.354531	0.895536	2.847726
Storage Temperature	24.08725	1	24.08725	48.58147	6.55E-06	4.60011
Interaction	4.220728	6	0.703455	1.418795	0.275157	2.847726
Within	6.94136	14	0.495811			
Total	36.30402	27				

Table 3.23 Phospho-Bound ANOVA

	Total	*Freezer*	*Room*
Count		14	14
Sum		198.2203	220.2895
Average		14.15859	15.73496
Variance		9.412338	2.204261

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	6.744765	6	1.124128	0.117975	0.992479	2.847726
Storage Temperature	17.39464	1	17.39464	1.825538	0.198088	4.60011
Interaction	10.87201	6	1.812002	0.190167	0.974552	2.847726
Within	133.399	14	9.5285			
Total	168.4104	27				

Table 3.24 Phospho-Total ANOVA

	Total	*Freezer*	*Room*
Count		14	14
Sum		335.2345	383.2738
Average		23.94532	27.3767
Variance		9.205337	3.77459

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	8.052132	6	1.342022	0.135144	0.989262	2.847726
Storage Temperature	82.42033	1	82.42033	8.299855	0.012089	4.60011
Interaction	21.66225	6	3.610375	0.36357	0.890011	2.847726
Within	139.0247	14	9.930334			
Total	251.1594	27				

Free Phospholipids (FPL) had significant differences for storage temp, but not days (Table 3.22). Frzr and RT had parallel, nearly horizontal slopes (Fig 3.11). However, the intensities were greater (and amounts greater) at all points for the RT samples (Fig 3.11).

Bound Phospholipids (BPL) did not show significant differences for storage temp or days (Table 3.23). However, it was interesting to note that the BPL Frzr samples had a larger standard deviation than did samples in other lipid classes. This was not the case for RT BPL. Day 12 appeared to have a high value for RT compared to the rest of the RT samples. Both RT and Frzr linear regressions had roughly horizontal slopes (Fig 3.12). Slopes for Bound Frzr and RT Phospholipids were approximately horizontal over storage time, but showed a slight increase over time. However, RT Free showed a slight decrease over storage time. Bound Day 12 had a large value for both duplicates. Without these larger values, it appears that RT Phospholipids might have had a very slight decrease over storage time for each of total, free, and bound.

Total Phospholipids (TPL) exhibited a significant difference between storage temps, but not days (Table 3.24). RT samples had generally higher intensity values than did Frzr (Fig 3.13). Another possible explanation was that the solvent for the RT sample had evaporated slightly while the sample was in solvent and the vial was being opened for transferring or dispensing sample and thus a more concentrated sample was applied to the TLC plate. Day 12 RT had uniquely high values, which were a result of high values for both the Free and Bound RT that made up the TPL. Both Frzr and RT had approximately horizontal slopes (Fig 3.13). There was no obvious experimental explanation for the high Day 12 free and bound RT values.

When evaluating phospholipids by storage temperature, horizontal to slightly positive slopes were apparent across storage time for both Frzr and RT Phospholipids at each of the total, free, and bound levels (Fig 3.9 and 3.10).

Figures 3.16 through 3.20 present the cumulative intensity in storage time of the three flour neutral lipid fractions (free, bound, and total) as a function of storage time. Separate slots on each figure show changes during frozen vs. room temperature flour storage.

Tables 3.25 through 3.27 are the ANOVAs that correspond to free, bound, and total neutral lipids, respectively.

Figure 3.16 Neutral-Free by storage condition over time

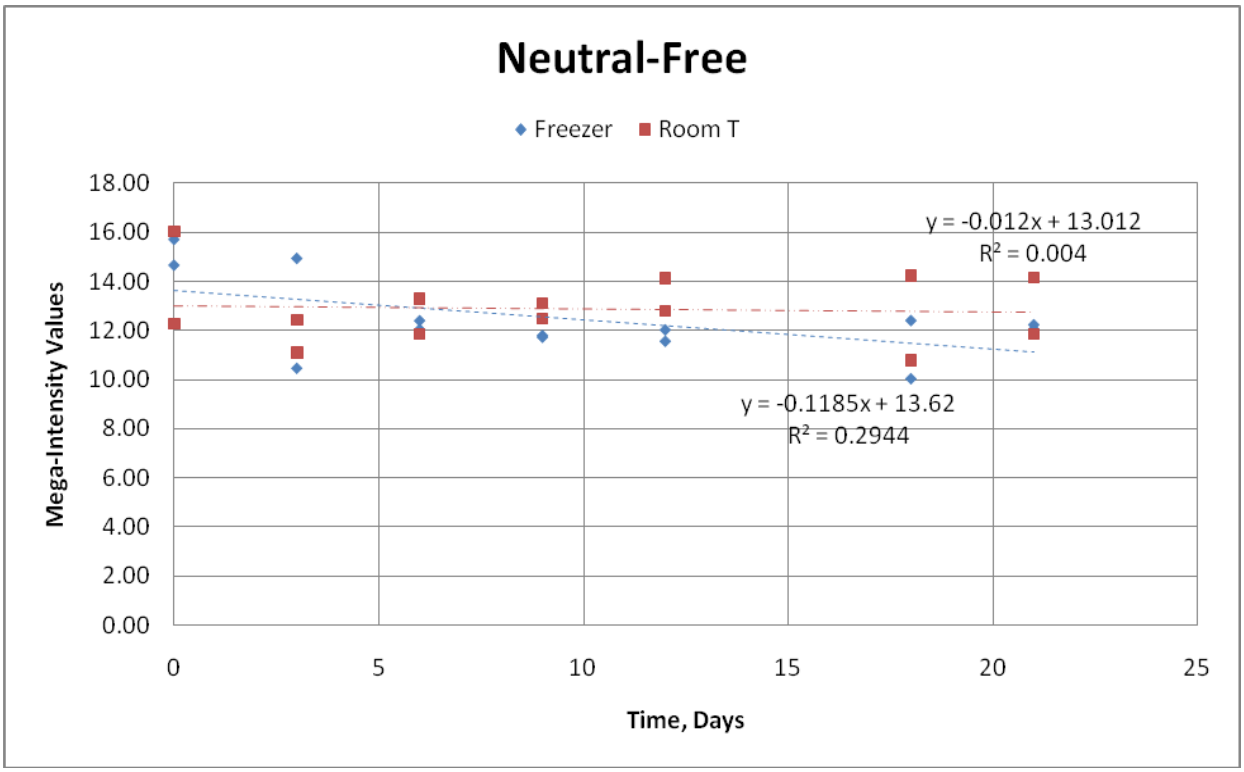


Figure 3.17 Neutral-Bound by storage condition over time

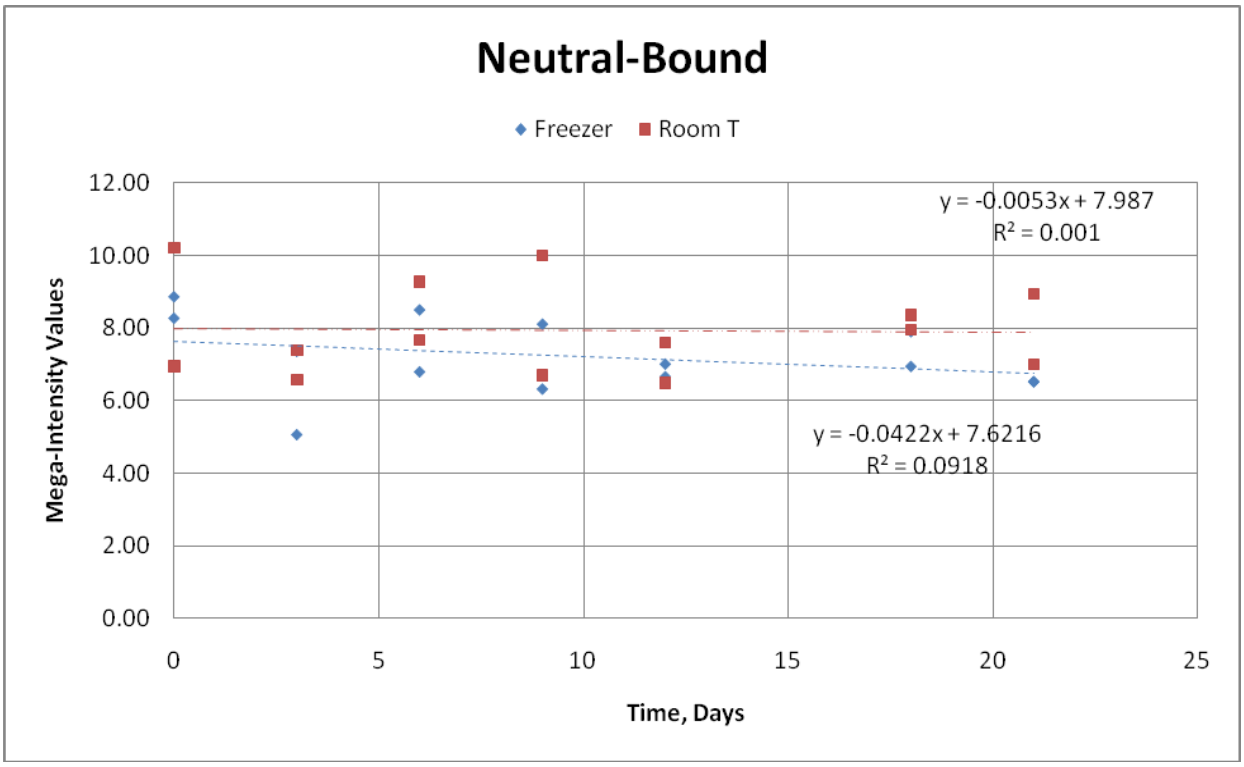


Figure 3.18 Neutral-Total by storage condition over time

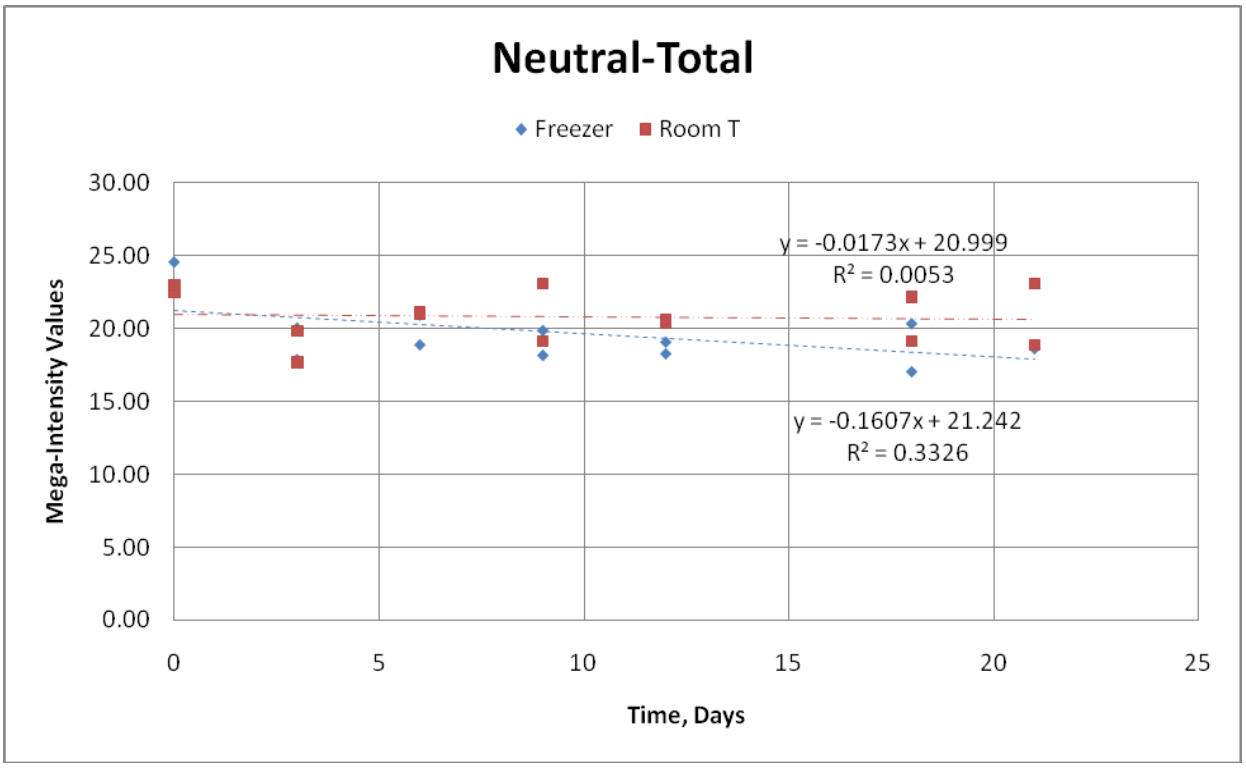


Figure 3.19 Neutral-Frzr by free, bound, total, over time

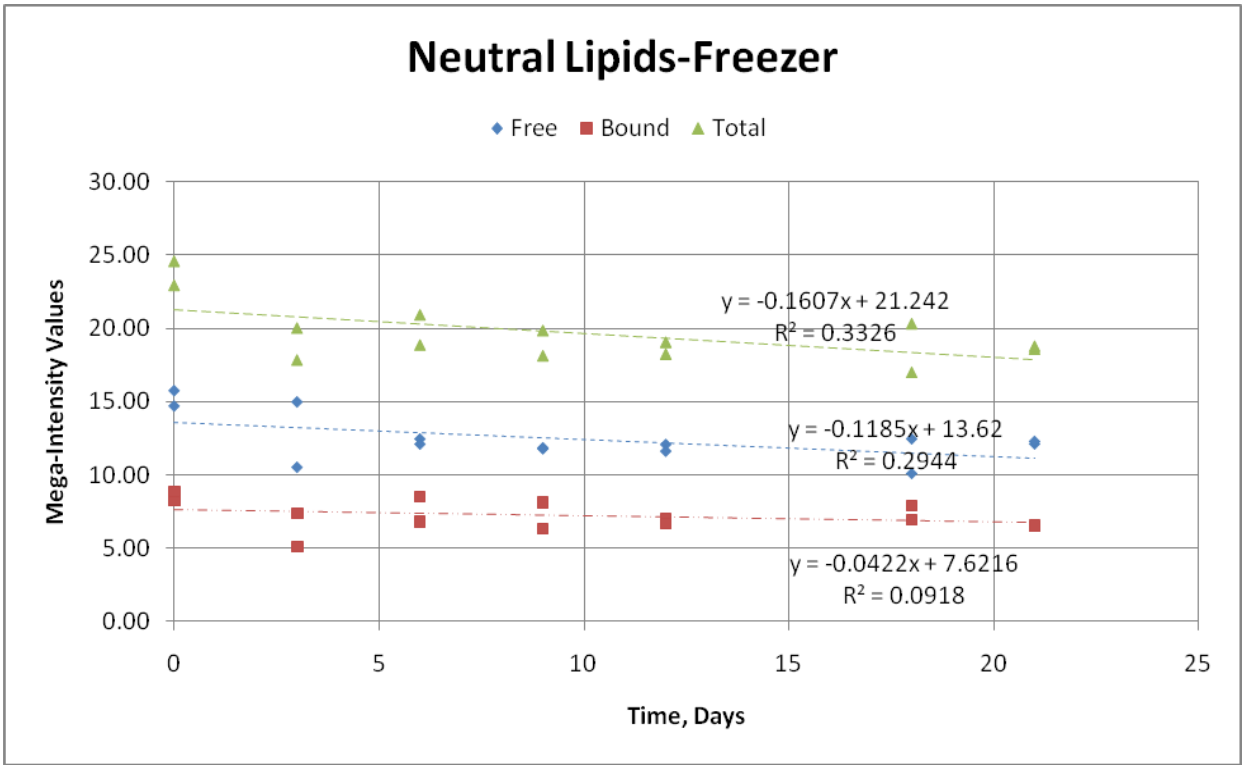


Figure 3.20 Neutral-RT by free, bound, total, over time

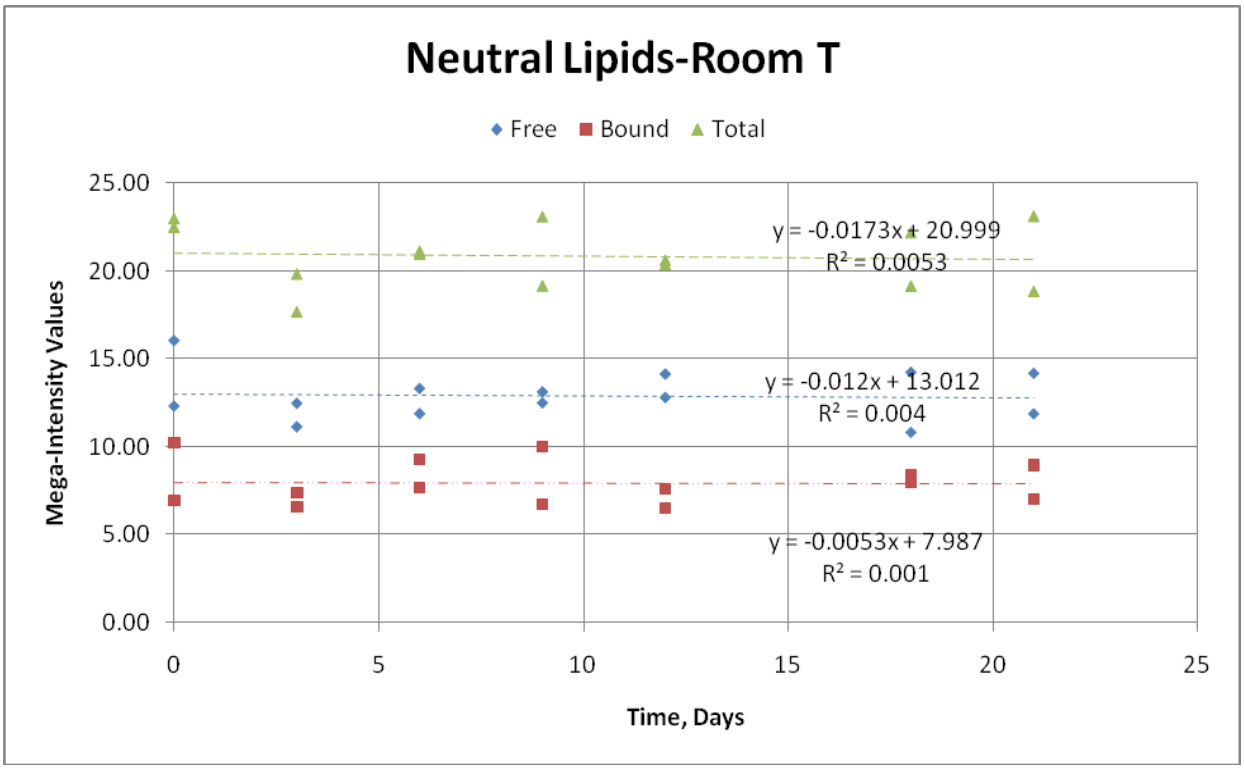


Table 3.25 Neutral-Free ANOVA

	Total	*Freezer*	*Room*
Count		14	14
Sum		174.331	180.5063
Average		12.45221	12.89331
Variance		2.602825	1.973342

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	20.44612	6	3.407687	1.475794	0.256161	2.847726
Storage Temperature	1.361943	1	1.361943	0.589828	0.455245	4.60011
Interaction	6.717319	6	1.119553	0.484854	0.808974	2.847726
Within	32.32674	14	2.309053			
Total	60.85212	27				

Table 3.26 Neutral-Bound ANOVA

	Total	*Freezer*	*Room*
Count	14	14	
Sum	100.8818	111.0881	
Average	7.205846	7.934861	
Variance	1.058054	1.554111	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	11.16226	6	1.860376	1.222131	0.352022	2.847726
Storage Temperature	3.720242	1	3.720242	2.443927	0.140297	4.60011
Interaction	1.484546	6	0.247424	0.16254	0.982775	2.847726
Within	21.31135	14	1.522239			
Total	37.67839	27				

Table 3.27 Neutral-Total ANOVA

	Total	*Freezer*	*Room*
Count	14	14	
Sum	275.2128	291.5944	
Average	19.65806	20.82817	
Variance	4.237113	3.109806	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Days	48.40048	6	8.066747	3.042188	0.040441	2.847726
Storage Temperature	9.584078	1	9.584078	3.614415	0.078066	4.60011
Interaction	9.986703	6	1.664451	0.627709	0.706082	2.847726
Within	37.12277	14	2.651626			
Total	105.094	27				

Free Neutral Lipids (FNL) were not significantly different for storage temp or days (Table 3.25). Frzr samples showed a slight decrease over time. RT samples had an

approximately horizontal slope, but with low values on Day 3 (Fig 3.16). No experimental explanation could be found for Day 3 values.

Bound Neutral Lipids did not vary significantly for storage temp or days (Table 3.26). Frzr samples showed a slight decrease over time, and had one low value on Day 3 (Fig 3.17). RT samples showed an approximately horizontal slope over time, however with Days 3 and 12 at lower values. No experimental explanation could be found for the lower values at Day 3 or Day 12.

Total Neutral Lipids (TNL) were found to be significantly different for days, but not storage temp (Table 3.27). Both storage conditions resulted in a slight decrease over time (Fig 3.18). This was more pronounced for Frzr, which is surprising as the cause was related to temperature. RT values remained constant over time, except for Day 3. It is interesting to note that on Day 3, Frzr and RT had the same values. Ideally, this would have happened for Day Zero samples. As mentioned above for FNL, no experimental explanation was apparent for the lower value of RT Day 3. However, for Frzr Bd Day 3, one value may have been lower than expected due to a small sample spill of Frzr Bd-B Day 3. A slight decrease over storage time was apparent for both Frzr and RT Neutral Lipids at each of the total, free, and bound levels (Figs 3.19 and 3.20).

Possible Explanations for Changes in Total Lipids

Several factors could be responsible for the slight increase observed over storage time. Larger amounts of sample or standards could have been dispensed on the TLC plates over time because of increased concentration of lipids due to evaporation of solvent as the standard was exposed to air during the experiment. Similarly, a number of factors could be responsible for a decrease over storage time of Total Lipids. Breakdown of lipids in a specific class over time could result in smaller spots on the TLC plate. Lipids in general may have become less extractable over time. Samples were only opened to apply to the TLC plate once; the same lipid standard was used during the several days of making TLC plates. It is possible that the sample concentration could be standardized using normalization of the lipid standard.

Other Possible Factors Affecting General Lipid Results

A number of other factors may have influenced the results. At two years of age, the wheat may already have aged prior to receiving, milling, baking, and chemical analysis. This

would limit the changes observed during this storage study. It is also possible that certain lipid classes are more subject to reactions than others. It may be possible that lipids were converted from free to bound or vice versa during the storage experiment. This could happen if bound lipids such as glycolipids or phospholipids were broken down by an enzyme into free fatty acids which change their solublility in certain solvents to categorize them as free lipids. However, flour moisture content was strictly controlled, in that the flour was stored in sealed plastic bags inside plastic bins. Storage of flour samples in virtually air-tight plastic bags may have limited some chemical reactions. Storage of flour in paper bags or in tins with headspace may have allowed more reactions to occur, or for reactions to occur at a faster rate.

Table 3.28 Statistically significant parameters according to ANOVA

Days	
C-Cell	TLC
Bread volume, specific volume: RT>Frzr Slice area: RT>Frzr Slice brightness: RT>Frzr Non-uniformity: RT>Frzr Wall thickness: Frzr>RT Cell diameter: Frzr>RT	Neutral Lipids Total

Limited comparisons can be made between C-Cell and TLC for Days (Table 3.28). C-Cell had many parameters which were statistically significant for Days. However, for TLC results, only total neutral lipids were statistically significant. The three graphs (Figures 3.16, 3.17, and 3.18) of neutral lipid (Free, Bound, and Total) displayed a slight decrease over storage time for Frzr neutral lipids, and virtually no change in the quantity of RT neutral lipids. However, this was only significant for total neutral lipids. It would make more sense if both total neutral lipids and either free or bound were both significant. It is possible that this result is due to unusually high or low values which were more evident in the cumulative values of total neutral lipids versus in the individual classes of free or bound neutral lipids. Days 3, 9, 18, and 21 of total neutral lipids had visibly larger standard deviations than days 0 or 12.

Table 3.29 ANOVA Statistically significant parameters for storage temp

Storage Temp	
C-Cell	TLC
Non-uniformity: RT>Frzr Cell diameter: Frzr>RT	Glycolipids Free Glycolipids Bound Glycolipids Total Phospholipids Free Phospholipids Total

C-Cell and TLC parameters which were statistically significant for storage temp are shown in Table 3.29. Changes in Glycolipids (free, bound, total) or Phospholipids (free, total) at RT may be related to decreased cell diameter. It could be that maturing or chemical changes during storage of flour at RT produced finer cells when baking, but did not produce more uniform cells.

Table 3.30 ANOVA statistically significant parameters for the interactions of Days*Temp

Interaction of Days*Temp	
C-Cell	TLC
Non-uniformity: RT>Frzr	None

For the interaction of Days*Temp, only non-uniformity was statistically significant for C-Cell (Table 3.30). The greater C-Cell non-uniformity at RT than Frzr at the interaction of Days and Temp did not appear to be related to any parameters of TLC.

CHAPTER 4 - Conclusions

A laboratory scale milling procedure was developed which successfully mimicked the granulation curves of commercially milled whole wheat flour. In addition, AACCI Method 10-10B (100 gm 'Pup' Loaf) was successfully modified and adapted for use in whole wheat flour baking analyses. Post milling storage studies of whole wheat flour baking quality did not identify any time or storage related trends in either loaf characteristics or lipid composition. However, because the wheat supplied for the studies was later discovered to be > 1 year old, this lack of trends is not definitive proof that post milling changes do not occur.

Suggestions for continued research include: using true (< 1 week from harvest) new crop wheats of both strong and intermediate baking characteristics, increasing the sampling/analysis rate during the first week of storage and extending the total sampling/storage time, analyzing for time and temperature associate changes in lipid hydrolytic enzymes (particularly galactosidases) and, using lipid profiling to obtain quantitative measures of changes in individual lipid species over storage time.

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Appendix A - C-Cell Parameter Definitions from C-Cell Instruction Manual

Interpretation of Processed Images

When C-Cell images are analysed, five processed images are produced, which highlight different aspects of the analysed slice. These are initially displayed in the Processed Images window.

The following image types are used:

Raw

This type of image displays the original, unprocessed image of the slice selected for analysis.

Cell

This type of image displays the individual cells within the product slice. Each one is colour coded according to its prominence, based on its area and depth, quantified by the 'volume' parameter. Small cells are coloured in dark blue and larger ones are shown in lighter shades of blue, green and yellow. Cells large enough to be classified as holes are outlined in red.

Volume

This type of image displays contours of the coarseness of the texture, based on volume measurements of cells. The coarsest 50% of the slice area is shaded in red and the finest regions are shaded in blue. The range of values displayed in this image are used for calculation of cell size measurements on the cell volume (map) scale. The shape of the red and blue regions is used for calculation of the Coarse/Fine Clustering measurement.

Brightness

This type of image shows a view of the slice, corrected to remove any differences in overall product reflectance. The image is shaded in brown to avoid confusion with the raw image. The image can be useful to enable structures in dark slices to be seen clearly on screen.

Elongation

This type of image represents the orientation and elongation of cells. Short red lines are drawn parallel to the long axis of cells at each point in the slice. The length of the lines indicates the degree of elongation of the cells. For regions that show some curvature, green lines are also drawn that point towards the centre of the curvature. The length of the green lines indicates the degree of local curvature. Yellow lines are also shown that divide regions of the slice that show curvature in opposite directions. Regions of the slice that show a complete 360 degree rotational structure are shaded in bright blue. Those that show a rotational structure that turns through 180 degrees are shaded in pale blue. Those showing no full rotation are left in grey.

Shape

This type of image shows a view of the slice with particular shape features shown in color. A white rectangular box is shown enclosing the slice. Measurements of cell position (available in the Advanced Edition) are made within the coordinate system defined by this box, and measurements of slice Height (max) and Breadth are given by the dimensions of the box. The corners of the slice are also identified and are connected by white lines to each other and to the centre of the slice. Concavities in the sides of the slice are shown in blue for the bottom, green for the sides and red for the top. Where oven spring is detected, this is shown in yellow. The points used for measuring the slice height are marked as yellow points on the top edge. High points are identified at either side of the top edge and the lowest point between them is also marked. Where there is no clear dip in the top, some of these points may coincide and it may not be possible to see three distinct points.

Interpretation of results

Results Shown

C-Cell measures approximately 50 values for each slice, designed to suit the varying requirements of different users and product types. In practice, individual users may not regularly require all the information, but may prefer to concentrate their attention on smaller sets of values relevant to a particular situation. The list of results displayed can be customized using the User Options. As far as possible, measurements are intended not to be specific to a particular product type. Instead, they provide a general description of product characteristics, which can be compared by the user to the requirements for individual product types. The measurements are grouped in the Results window and in printed reports in the following categories:

Slice Information

- Dimension
- Brightness
- Shape

Cell Information

- Cell Size
- Cell Elongation and Orientation

  Definitions of each measurement are given below.

Dimension

This section provides information on the overall dimensions of the product slice. Measurements of linear dimensions are displayed in units of pixels or millimeters, depending on the option selected in the User Options.

The height and breadth of the slice are defined with reference to the slice orientation specified prior to analysis. The base of a slice is identified as an edge on which the slice could sit squarely without tipping over. The vertical axis of a slice is taken perpendicular to this edge. The orientation identified can be seen in the Shape image. Note: For unusually shaped products, the point of stable balance may be quite far from the orientation originally indicated by the user.

Measurement	Description
Slice Area	The total area of a product slice.

Brightness

This section provides information related to the levels of overall brightness in the product slice.

Measurement	Description
Slice Brightness	The mean grey level (0-255) of pixels within the slice. The value is lower for products with a darker crumb (e.g. wholemeal bread as compared with white bread), and for products with larger or deeper cells that contribute to greater shadows. The measurement provides a useful indication of product reflectance. However, for more detailed assessment of this product characteristic, reflectance meters may provide a more accurate measurement.

Shape

Shape measurements are based on the shape of the slice perimeter. They include measurements of the concavity of the slice edges, the dimensions and presence of a break, and the roundness of the slice corners. With the exception of break height, depth and position, shape measurements are dimensionless values, expressed as percentages, and are intended to be independent of product size.

Concavity measurements are based on the shortfall of each slice edge from a curve tangent to the slice. This may indicate the presence of features such as side wall collapse, or ‘keyholing’, or may be a natural feature of the intended product shape.

For some types of bread product, a break in the side crusts may be formed during baking, and is often associated with oven spring. This is often visible as a less strongly colored region of crust. For sliced products, as used by C-Cell, this phenomenon may also result in an indentation in the side wall of the product, usually towards the top. C-Cell attempts to detect this indentation on each side of the product, and measures the size and position of this break. The break is shaded in yellow in the Shape image. If no break is detected on a particular side of the product, all the break measurements for this side are set to zero. For product types not normally associated with oven spring or a break, C-Cell will still attempt to detect an indentation towards the top of each

side of the product, but the interpretation of the measurement will be different. Shoulder or Roundness measurements describe the roundedness of the product corners. The shoulder is measured considering imaginary tangents to the adjacent product sides, drawn close to the corner. The area enclosed between these tangents and the edge of the slice is measured and expressed as a percentage of the slice area.

Cell Size

For each cell in the slice, two main size parameters are calculated - area and volume:

- The area of a cell is based on the total number of pixels within it, either expressed as the number of pixels, or multiplied by the area of one pixel to give a result in units of mm^2 .
- The volume measurement of a cell combines information on its area and its depth, based on the degree of shadow within it. It is not a true measurement of the physical volume of the cell. However, like true volume, it is higher for cells with larger area or greater depth. The relative volume of each cell is indicated by its color in the Cell image.

The Special Edition of the software enables a list of the area measurements of each cell to be output. The Advanced Edition also enables a list of the corresponding volume measurements to be output.

Based on the individual measurements of all the cells in a slice, several statistics are calculated, indicating aspects such as the average size of cells and the range of sizes present. In the Special and Advanced Editions, a chart showing the cell size distribution can be included in printed reports.

In addition to measurements based directly on individual cell measurements, C-Cell generates a smoothed map of the distribution of cell volumes at each point in the slice, displayed as the Volume Contours image. Some statistics are also calculated based on the range of volumes in this contour map. Volume statistics calculated on this basis are indicated by the suffix "(map)", and are represented on a different measurement scale, where:

Volume (map) = 1 + 3.5 x volume

Cells with volume measurements much greater than those typical of the measured slice are classified as holes. These are identified in the Cell image by red outlines. Holes are included within measurements of overall cell structure, but additional measurements are also provided that relate to these cells alone. The size at which a cell would be considered a hole depends on the

product type. To provide flexibility for measurement of a wide range of products, C-Cell does not therefore use a fixed threshold volume for classification of holes, but instead adapts this to the cell structure of the product being measured. The sensitivity to holes can be controlled by adjusting the Hole Size Sensitivity parameter in the Advanced Settings window. For comparability with results obtained by other users, it is recommended that users should standardise on the default value of 4.50 for this parameter.

Note: For products that have been poorly sliced, or which do not lie flat within the imaging cabinet, depressions in the slice surface may cast shadows and be identified as large, shallow holes. Poor slicing of products is also likely to have a strong effect on other C-Cell measurements.

Definitions of the individual cell size measurements are given below:

Measurement	**Description**
Number of Cells	The number of discrete cells detected within the slice. Higher values may be due to a finer structure or a larger total slice area. The cells are shown in the Cell image. When interpreting this image, cells only touching diagonally are considered to be discrete.
Cell Diameter	The average diameter of cells (pixels), based on measurements of the average cell area. This is a good general purpose indicator of the coarseness of the texture, but does not take the depth of cells into account. Cell Volume provides an alternative measurement that also includes consideration of cell depth.
Wall Thickness	The average thickness of cell walls. For bright slices, saturation of some regions may be interpreted as thick walls. Walls close to the edge of the slice are given a reduced weighting in the calculation.
Non-Uniformity	A measure of the lack of uniformity between fine and coarse texture (including holes) across the slice. High values indicate less uniformity of texture. The value is useful for comparing slices of similar types of product, but comparisons between products of differing type tend to be less easily interpreted.

Cell Elongation and Orientation

Cells in products may be elongated. For each cell, C-Cell measures the direction of orientation parallel to the longest axis. The elongation is defined as the ratio of the length of the cell measured in this direction to the breadth measured in a perpendicular direction. The elongation of a cell is always greater than or equal to 1.

The Advanced Edition provides the facility to output the elongations of individual cells. Based on the orientation and elongation of individual cells, C-Cell calculates average elongation and orientation values for the whole slice. It also identifies and measures particular patterns of cell orientation, such as vertical alignment of cells, or a circulating structure. The local cell orientation and elongation at each point in the slice is shown in the Elongation image.

Details of the individual elongation and orientation measurements are given below:

Measurement	**Description**
Average Cell Elongation	The average length to breadth ratio of cells, independent of their relative orientation. Lower weighting is given to cells close to the edge of the slice. Values close to 1 indicate rounded cells. Higher values indicate greater elongation.
Cell Angle to Vertical	The angle of the direction of Net Cell Elongation, measured clockwise from the slice vertical. Lower weighting is given to cells close to the edge of the slice. Values are given in the range of -90 to +90 degrees. Values close to 0 represent a vertical orientation. Values close to ±90 represent a horizontal orientation. If the Net Cell Elongation is low, it should be recognized that the orientation refers to a structure with little overall elongation, and therefore may be of limited significance.

Appendix B - C-Cell Parameter Definitions from C-Cell Instruction Manual

Table 4.1 C-Cell pup loaf bread slice data from 21-day baking of Karl 92 freezer (F) and room temp (R) stored flour samples

Day	Slice Name	Storage Temp	Slice Position	Slice Area (mm²)	Slice Brightness	Number of Cells	Wall Thickness (mm)	Cell Diameter (mm)	Non-Uniformity	Average Cell Elongation	Cell Angle to Vertical (°)
0	1	F	C	5016	88.2	3299	0.428	1.967	1.477	1.50	-1.6
0	3	F	C	5285	87.2	3311	0.430	2.096	2.150	1.53	2.2
0	5	F	C	4870	86.4	2946	0.440	2.108	2.106	1.53	-0.3
0	7	F	C	4964	86.1	2983	0.449	2.078	1.806	1.51	2.8
0	9	F	C	5185	88.7	3283	0.428	2.066	1.346	1.53	4.6
0	11	F	C	5356	89.6	3452	0.425	1.986	1.714	1.58	-0.5
0	2	R	C	4931	89.6	3115	0.436	2.035	1.648	1.50	2.4
0	4	R	C	5004	87.9	3155	0.431	1.973	15.284	1.53	1.9
0	6	R	C	4997	85.5	2950	0.444	2.160	15.390	1.48	0.7
0	8	R	C	4950	89.9	3326	0.425	1.979	9.478	1.50	-1.6
0	10	R	C	5148	88.2	3286	0.434	1.902	3.127	1.53	-6.5
0	12	R	C	5191	87.7	3300	0.431	1.967	1.498	1.53	-5.3
3	4	F	C	4836	88.9	3125	0.433	2.066	2.044	1.51	1.8
3	5	F	C	4732	90.7	2962	0.435	2.066	1.462	1.51	1.0
3	7	F	C	5130	92.0	3322	0.427	2.017	0.900	1.52	1.0
3	8	F	C	4963	89.0	3123	0.434	1.986	1.176	1.52	4.4
3	9	F	C	4763	89.2	3001	0.431	2.060	2.950	1.51	7.7
3	10	F	C	5209	88.7	3197	0.437	2.084	2.858	1.53	-1.4
3	1	R	C	5067	90.9	3371	0.428	2.004	1.412	1.52	-0.6
3	2	R	C	5027	88.6	3289	0.424	1.842	0.845	1.52	-1.4
3	3	R	C	5015	91.0	3396	0.423	1.998	2.671	1.50	7.4
3	6	R	C	5212	90.3	3231	0.431	2.166	4.212	1.52	7.0
3	11	R	C	5084	90.1	3265	0.427	1.882	1.431	1.54	2.2
3	12	R	C	4979	90.0	3296	0.425	1.922	1.581	1.52	1.0
6	1	F	C	4574	86.2	3038	0.425	1.876	1.196	1.52	-5.4
6	3	F	C	5185	90.5	3366	0.427	2.035	1.222	1.55	0.5

6	5	F	C	5075	87.7	3217	0.432	2.035	1.298	1.55	-1.0
6	7	F	C	4975	87.8	3159	0.427	2.078	1.007	1.51	14.5
6	9	F	C	4983	88.8	3352	0.419	1.896	1.147	1.48	11.1
6	12	F	C	5186	89.7	3456	0.425	2.029	1.772	1.53	-4.9
6	2	R	C	4975	87.9	3157	0.434	2.041	2.470	1.53	-2.2
6	4	R	C	5009	88.4	3235	0.428	1.967	1.494	1.52	11.6
6	6	R	C	4954	88.5	3198	0.430	1.954	0.937	1.52	-3.7
6	8	R	C	4743	89.9	3111	0.427	1.915	2.459	1.49	0.6
6	10	R	C	5132	88.4	3072	0.445	2.066	1.937	1.51	-4.3
6	11	R	C	5173	90.1	3315	0.429	2.048	16.837	1.53	-1.1
9	2	F	C	4905	88.0	3125	0.435	2.054	1.802	1.53	-1.3
9	4	F	C	4248	83.7	2395	0.456	2.143	2.411	1.51	5.9
9	6	F	C	4706	87.3	3037	0.434	2.060	2.060	1.54	-1.5
9	7	F	C	4756	88.6	2932	0.437	2.108	2.246	1.53	1.6
9	8	F	C	5130	90.4	3273	0.436	2.017	1.204	1.51	4.8
9	11	F	C	4355	85.8	2484	0.456	2.131	2.272	1.53	6.8
9	1	R	C	4890	90.3	3117	0.436	2.035	0.954	1.52	-3.1
9	3	R	C	5194	89.2	3248	0.437	2.054	1.950	1.51	0.4
9	5	R	C	4708	88.2	2858	0.447	2.041	1.399	1.54	2.0
9	9	R	C	4932	94.3	3179	0.433	1.973	1.427	1.50	-1.2
9	10	R	C	5105	91.2	3317	0.427	1.992	1.482	1.54	-0.2
9	12	R	C	5056	90.2	3192	0.433	1.960	1.143	1.51	1.3
12	3	F	C	5293	90.1	3414	0.429	2.023	1.520	1.53	-3.1
12	5	F	C	5377	87.5	3530	0.424	2.004	0.983	1.55	-0.2
12	6	F	C	5367	89.3	3322	0.435	2.072	1.443	1.52	9.0
12		F									
12	10	F	C	4380	87.7	2548	0.451	2.078	1.121	1.54	-3.8
12	11	F	C	5054	88.6	3294	0.433	1.967	1.158	1.51	7.0
12	1	R	C	4847	88.6	3125	0.433	2.029	1.378	1.52	2.3
12	2	R	C	5276	88.5	3355	0.429	2.011	1.276	1.54	-3.1
12	4	R	C	4972	87.8	3141	0.435	2.131	1.407	1.53	0.0
12	7	R	C	4907	87.8	3087	0.436	2.029	3.036	1.53	5.3
12	9	R	C	5123	90.0	3311	0.428	1.986	1.120	1.50	2.7
12	12	R	C	5222	88.4	3350	0.433	1.998	4.971	1.53	0.5
15	1	F	C	5196	88.1	3291	0.433	2.114	1.038	1.51	-0.9
15	3	F	C	5477	85.4	3477	0.431	2.119	1.204	1.54	-0.2

15	4	F	C	5190	90.0	3215	0.436	2.072	1.052	1.53	-2.4
15	5	F	C	4978	89.5	3094	0.432	2.078	0.547	1.54	-2.0
15	9	F									
15	10	F	C	5269	89.1	3172	0.443	2.217	1.704	1.52	0.8
15	2	R	C	4759	86.2	2930	0.438	2.108	1.332	1.53	-2.4
15	6	R	C	5296	89.5	3445	0.423	1.998	1.597	1.57	7.3
15	7	R	C	5119	90.0	3137	0.436	2.078	1.859	1.54	-2.6
15	8	R	C	5185	90.3	3436	0.423	1.960	1.032	1.56	1.5
15	11	R	C	4978	86.5	2827	0.454	2.327	1.293	1.51	1.5
15	12	R	C	5322	90.9	3346	0.442	2.023	1.379	1.48	15.5
18	1	F	C	4878	88.4	3060	0.430	1.992	5.181	1.54	-0.6
18	3	F	C	4933	91.1	3155	0.432	2.011	1.789	1.53	4.8
18	4	F	C	5062	89.7	3209	0.427	1.998	1.063	1.57	3.2
18	6	F	C	5157	92.0	3173	0.432	2.054	1.042	1.57	-1.3
18	8	F	C	5183	92.9	3405	0.423	1.935	1.327	1.54	3.6
18	10	F	C	5009	94.1	3180	0.429	1.998	1.765	1.50	-2.7
18	2	R	C	4792	86.7	2925	0.436	2.004	1.210	1.55	2.9
18	5	R	C	5023	89.6	3301	0.423	1.935	0.683	1.52	0.8
18	7	R	C	4905	92.6	3263	0.426	1.909	0.518	1.51	7.5
18	9	R	C	4891	94.1	3294	0.422	1.869	1.195	1.50	0.0
18	11	R	C	5115	92.6	3355	0.431	1.992	1.318	1.54	3.6
18	12	R	C	4996	94.0	3250	0.424	1.986	1.161	1.53	-1.8
21	1	F	C	5158	87.8	3154	0.437	2.066	0.503	1.54	5.9
21	2	F	C	5345	92.3	3405	0.428	2.004	1.592	1.53	9.2
21	4	F	C	4890	88.6	2973	0.441	2.084	4.168	1.56	0.5
21	5	F	C	5155	89.2	3177	0.439	2.023	1.854	1.51	3.7
21	8	F	C	5006	90.9	3157	0.432	1.948	1.305	1.54	-1.9
21	11	F	C	4806	91.8	2783	0.458	2.234	2.930	1.47	3.3
21	3	R	C	4996	88.6	3187	0.429	1.979	1.247	1.55	5.0
21	6	R	C	5182	90.6	3261	0.440	1.915	1.349	1.51	26.7
21	7	R	C	5006	85.9	3111	0.433	1.992	2.672	1.54	3.3
21	9	R	C	5174	90.1	3367	0.422	1.992	1.373	1.54	-2.6
21	10	R	C	5490	91.3	3439	0.433	2.017	2.129	1.58	-1.1
21	12	R	C	5281	91.8	3086	0.443	2.217	2.338	1.58	12.3