EFFECT OF ENZYME APPLICATION IN TEMPER WATER ON WHEAT MILLING

by

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Abstract

The effect of enzyme in temper water on wheat milling performance and flour quality was studied. Five independent variables, enzyme concentration, incubation time, incubation temperature, tempered wheat moisture content, and tempering water pH, were studied. An enzyme cocktail consisting of cellulase, xylanase, and pectinase was used at 5 different concentrations. A single pure variety of hard red winter wheat was tempered under defined conditions following an RSM central composite design which required 33 tests including 7 replicates. Each treatment had 5 levels: high, medium high, medium, medium low, and low. After tempering, the physical characteristics of the wheat kernel were determined by using the Single Kernel Characterization System. An experimental laboratory mill (Ross Mill) was used to mill wheat into flour. Thirteen streams of flour, and additional streams of bran, shorts, red dog, and germ were obtained. Product yield, protein, ash, and flour color were evaluated. The data were analyzed and compared using the software SAS and RSM Plus.

The data showed that incubation time was the only significant factor affecting the tempered wheat hardness ($p<0.05$). The treatments affected the flour yield from the break rolls more than that from the reduction rolls. However, a maximum point for flour yield was not found. The relationship between treatments and flour yield was established with a prediction model equation. Also, the enzyme effect on the dough properties and bread making were investigated. The treatments did not affect the optimum water absorption for the flours. However, enzyme treated flours showed shorter mixing times. Regardless of the differences in mixing times, the specific loaf volumes were not significantly different for the all treatments.

Bread baked from the flour milled from enzyme treated wheat did not show a positive effect on bread staling.

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I would like to thank my family, Hoseok Song, and friends in Korea for their support. I know that you were praying for me and supporting me in somewhere. Thank you.

Dedication

This dissertation is dedicated to my family.

CHAPTER 1 - Introduction

Milling is a process by which cereals such as wheat are reduced in particle size so as to produce flour. Wheat milling consists of grain cleaning, tempering, grinding (break system and reduction system), and size separation. The ground particle size depends on the end-use and the type of products. Tempering means the addition of water or sometimes removal of water followed by a rest period (Posner and Hibbs, 1997). The unique feature of wheat that makes milling possible is that the three parts of the kernel (bran, germ, and endosperm) differ in relative toughness or friability.

The purpose of tempering is to toughen the bran, so it can resist being broken into small pieces, and to mellow the endosperm and make it easier to grind. One of the main goals in wheat tempering before milling is to distribute water in the kernel as uniformly as possible. Tempering is considered a very important stage for the miller from technical, flour quality, and economic points of view. The amount of water added varies with the original moisture content of the wheat, the relative humidity in the mill, and the desired moisture content for grinding. The resting time between tempering and milling can be determined from the rate of water diffusion throughout the whole kernel. The break system in the mill is very sensitive to variations in tempering moisture in the kernel from the optimum level. Break flour from low-moisture wheat has higher ash values, which is undesirable.

In hard red winter wheat, the starch endosperm (potential flour) amounts to 81.4 to 84.1% (dry matter basis) of the wheat kernel (Hinton, 1959). Despite the complexity of the conventional milling process, the normal commercial extraction rate is 70 to 77% (Jones and Ziegler, 1964). In the last decade or so, research efforts were made in various operations related to the milling process in order to separate bran more easily and effectively. The Satake Company developed a mechanical wheat debranning process, but the reception from the industry for this process was somewhat cold or luke-warm at best, because of its excessive energy need and questionable effectiveness in efficient bran removal.

For a long time, wheat milling has been accomplished by mechanical methods, but they have two disadvantages: 1) the loss of nutritional components which ideally would be recovered

and included in the flour without causing detrimental effects on flour quality, and 2) there is a large portion of endosperm left attached to the wheat bran. Although low moisture content tempered wheat and hard grinding can increase the flour extraction, it results in undesirable quality parameters such as high ash content and dark color. Therefore, as an alternative, the modification in chemical composition or physical structure of wheat can be seriously considered. Recently, enzymes have been introduced to various industries as solutions to many problems and there are many ongoing research projects regarding enzyme applications to maximize the positive effect in the industries. The uses of enzymes during tempering and their effects on the efficacy and efficiency of the milling process have not yet been established. The effort to optimize the condition for the maximum enzyme activity has not yet been published. From the viewpoints of improving the process, enhancing profitability to the cereal miller, human health benefits, and food safety, it is desirable that we research alternative methods for bran separation.

CHAPTER 2 - Literature Review

2.1. Wheat structure and composition

Wheat grain (Figure 2.1) consists of bran, endosperm, and germ. The percentages of each part of grain in the wheat kernel are shown in Table 2.1. Bran consists of pericarp and aleurone.

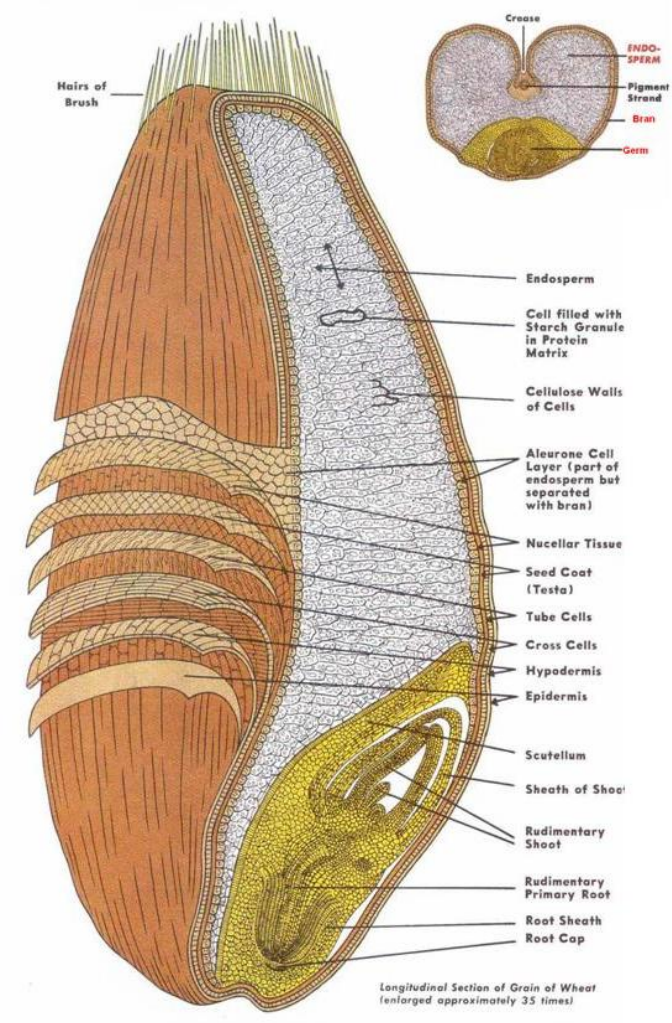


Figure 2.1 Longitudinal and cross sections of wheat kernel (adapted from Hoseney, 1998)

Table 2.1 The parts of grain in wheat kernels

Part of grain	Percentage of kernel weight
Bran	(15.0)
Pericarp	8.0
Aleurone	7.0
Germ	(2.5)
Embryo	1.0
Scutellum	1.5
Starchy endosperm	(82.5)
Outer	12.5
Middle	12.5
Inner	57.5
Whole grain	100

Adapted from Hinton (1953)

2.1.1. Aleurone

As shown in Table 1, aleurone takes up a large portion in the wheat kernel. Aleurone makes up to 7 to 9% (w/w) of the kernel, and 45 to 50% of the bran fraction. The aleurone layer (Figure 2.2) consists of living tissue, generally one cell thick, completely surrounding the kernel, covering both the starchy endosperm and the germ. From the botanical standpoint, it is the outer layer of endosperm (Hoseney, 1998). However, as it is removed during milling, it constitutes the innermost layer of bran. The aleurone layer is presumed to play a significant role in the milling process in that the separation of bran from starchy endosperm occurs near the aleurone layer interface where there is a different structure and biochemical composition from the rest of the endosperm. Aleurone contains most of the nutrients and physiological benefits of whole wheat but in a highly concentrated form. Table 2.2 shows the composition of wheat aleurone. Protein, dietary fiber, & lipid rich components are encased within the living aleurone cells. Aleurone cell walls are very strong, so as a result, they are not normally broken during the conventional milling process. The upper digestive tract also cannot digest the aleurone cells because of their very strong walls. As a result, the nutrients encased in the cells are not available to the body until they reach the large intestine. At that point, high-quality proteins, lipids, and B-vitamins are

released for absorption and digestion (Buri et al. 2004). For this reason, there are many ongoing research projects attempting to add value to the bran, or more precisely to the aleurone.

Greffeuille and coworker (2004) studied the distribution of the aleurone layer during the common wheat milling process by using the biochemical marker identification. Two molecular markers, phytic and para-coumaric (*p*-CA) acids, were used to quantify and describe the fate of the aleurone layer cellular content or cell walls in flour streams and total flour respectively. They found that the aleurone cellular content and the cell walls showed distinct fates in the milling process. The aleurone cell walls were released at the first break stage and found mostly in the break flours, whereas cellular content remained at the same level through the break stages to the reducing stages. Any damage to the aleurone cell walls is followed by release of the cellular content, so the flour after the break stage, therefore, must be enriched in aleurone cellular content, which means that the mode of grain rupture has a significant influence on the flour composition and nutrition.

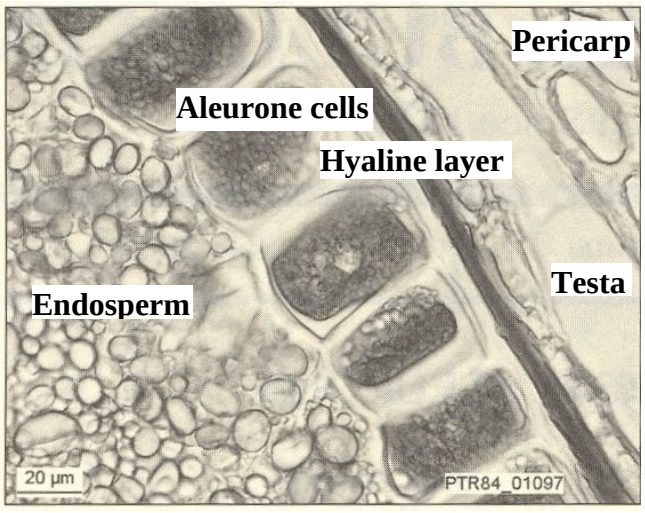


Figure 2.2 Microscopic view of the aleurone layer
(adapted from Buri et al. 2004)

Peyron and coworker (2003) focused on the characteristics of the aleurone layer and its association with wheat milling behavior. They suggested that there were two possible factors influencing the milling properties, which were the variability in thickness of the aleurone layer and the irregularity in shape of aleurone cells. Concerning the thickness and irregularity of the aleurone layer, large variations within grains and between grains from the distinct wheat samples

were observed. Consequently, it was found that the thickness of the aleurone layer and the structure of the aleurone did not significantly affect wheat milling behavior. Furthermore, the necessity of an investigation of the tissue adhesion mechanism on the aleurone and subaleurone layer interface was emphasized. They also hypothesized that the concentration and distribution of ferulic acid dehydrodimers in cells could be a factor which controls tissue adhesion.

Table 2.2 Wheat aleurone composition

Constituent	Method	Unit	Content
Crude protein, N×5.70	Leco	g/100g DM[a]	20.8
Crude Fat	Soxhlet	g/100g DM	5.7
Polyunsaturated fatty acids	HPLC fatty acid	% of crude fat	66
Monounsaturated fatty acid	Spectrum	% of crude fat	18
Saturated fatty acid		% of crude fat	16
Total Dietary Fiber	Enzymatic gavimetric method[b]	g/100g DM	47.1
Water insoluble dietary fiber		g/100g DM	43.0
Water soluble dietary fiber		g/100g DM	4.1
Crude Ash	Ashing oven, 590°C	g/100g DM	11.3
Phosphorus		g/kg DM	25.4
Potassium		g/kg DM	22.5
Magnesium	Microwaves-Mineralization+AAS	g/kg DM	10.3
Calcium		mg/kg DM	930
Iron		mg/kg DM	260
Zinc		mg/kg DM	139
Sodium		mg/kg DM	21
Vitamins			
B1 (Thiamin)	Swiss Manual of Food analysis (2002)	mg/100g DM	1.4
B2 (Riboflavin)		mg/100g DM	0.2
B6 (Pyridoxine)		mg/100g DM	1.3
Niacin		mg/100g DM	32.9
Folic Acid		mg/100g DM	158
Pantothenic Acid		mg/100g DM	4.9
E (DL-α-Tocopherol-AC)		mg/100g DM	1.2
Phytic Acid (4,5,6-IP)	Egli (2001)	g/100g DM	8.4

[a] DM
[b] AOAC method 991.43.
[C] Adapted from Buri et al (2004)

2.1.2 Cell Walls of Cereal Grain

The mature cereal grain is composed of the characterized tissues. For example, through the growth period, the cell contents of pericarp-seed coat disappear and only thick cell walls remain. In the case of starchy endosperm, thin cell walls are dead and packed with starchy granules and storage protein. In contrast, the cells of the aleurone, which are alive, have thick bilayered walls (3-5μM) containing the protein and lipid-rich cell contents (Stone, 2006). The cell wall components and composition of various grain tissues are different and have a significant influence on the end uses of grains. According to the composition and structure of grain tissues, the grain show different milling behaviors, such as water uptake during conditioning step and grinding action during break and reduction step. Plant cell walls consist of two different types of cell walls, primary and secondary. Primary and secondary walls contain different proportions of cellulose, hemicellulose, and pectin (Figure 2.3). The cellulose fibrils are embedded in a network of hemicellulose and pectin.

Aleurone cell walls show unique and far less complex structures than any of the other plant cell walls that have been studied, in that such a large percentage of its structural polymer consist of arabinoxylans. McNeil and coworkers (1975) studied the barley aleurone cell wall and found that aleurone cell walls were composed predominantly of two polysaccharides, arabinoxylan and cellulose, and protein, at 85%, 8%, and 6%, respectively. It has been well known that the aleurone layer is a secretory tissue, although the intact aleurone cell walls function as a barrier to the mobilization of secreted enzymes (McNeil, et al. 1975). In 1976, Taiz and Honigman focused on the extensive aleurone cell wall degradation during the tissue's response to gibberellic acid and concluded that three arabinoxylan-degrading enzymes – endoxylanase, arabinosidase, and xylosidase – increased in total activity and were released into the medium, aleurone cell wall, in response to gibberellic acid. Of these enzymes, only endoxylanase would be expected to degrade the intact polymer. Most of the arabinoxylans in the aleurone cell walls consist of a linear xylan backbone substituted with single arabinofuranosyl residues. It is thought that strong noncovalent bonds exist between the arabinoxylan chains themselves, and between arabinoxylan chains and cellulose fiber, which make up the network in the cell walls. The cellulose fibers embedded in this network contribute to the strength of the cell wall, but the protein in the cell walls may not be a structural component.

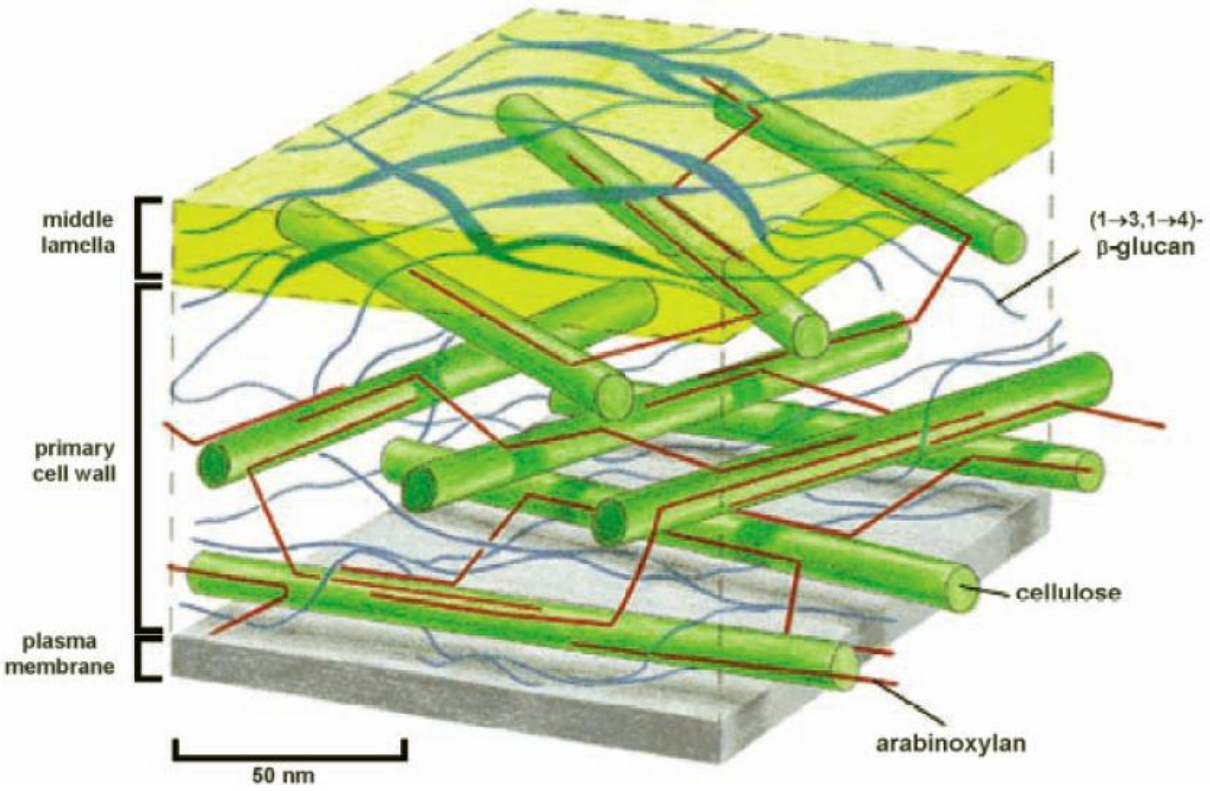


Figure 2.3 Simplified schematic representation of the spatial arrangement of polymers in a primary cereal wall of a cereal, e.g., the wall of starchy endosperm cell.
Primary cell walls are composed of cellulosic microfibrils and non-cellulosic matrix polysaccharides (hemicellulose: arabinoxylan, (1→3,1→4)-β-glucan)or glucomannan). The cellulose fibrils are embedded in a network of hemicellulose and contribute to the strength of cell walls.

2.2. Enzymes

Enzymes have been used in food processes such as beer, wine, bread, and cheese making for centuries, and the history of enzyme uses must be longer than recorded history. In spite of its long history, most advances in enzymology and microbiology have only occurred during the past century, resulting in more progress in enzyme production and application. As the advantages in using enzymes in food processing started to gain attention, the commercial enzyme applications have grown from a relatively insignificant role to one of the most important aspects of food

processing during the past half of a century. How many enzymes may exist is unknown, but it is thought that there are 10,000 or more. (Underkofler, 1972)

2.2.1 Carbohydrases

Cellulases

Cellulases break down cellulose to beta-glucose.

Xylanases

Xylanases degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants.

Pectinases

Pectinases break down the pectin to pectinic acid and finally pectic acid.

2.2.2. Factors affecting enzyme reactions

There are numerous factors affecting the enzyme action, and these factors should be considered for the enzyme applications.

Concentration of enzyme

For most enzymatic reactions the rate of the reaction is directly proportional to the concentration of enzyme, at least during the early stage of the reaction.

Concentration of substrate

The rate of enzyme reaction is proportional to the substrate concentration at very low substrate concentration. With an excess amount of substrate, the extent of enzyme reaction shows a linear relationship between the reaction time and the amount of product formed during the early stage of the reaction. As the enzymatic reactions proceed, the amounts of substrate decrease, which causes the reaction to slow down.

pH

pH has a significant influence on its activity due to the protein nature of enzymes. The optimum pH at which enzymes show the highest activity vary widely with the enzyme. Enzyme activities decrease rapidly above or below the optimum pH until the enzymes are completely

denatured and inactivated. Also, there is a pH range at which enzymes are most stable, which does not always coincide with the optimum pH.

Time

As mentioned above, the reaction rates slow down during the course of the enzymatic reaction due to many factors such as a reduction in the amount of substrate available or the inhibitive action of end products. Therefore, it is important in enzyme applications that sufficient time be allowed for the enzyme reactions to approach completion.

Temperature

Optimum temperature is another important factor affecting enzymatic reactions in two different ways. One effect is inactivation. Enzymes are denatured at high temperatures as well as at extremely low temperatures, losing their ability to catalyze reactions. The other effect is an acceleration of the reaction rate at high temperatures. Like most chemical reactions, the rate of an enzyme reaction increases with increasing temperature, up to a certain point, known as the optimum temperature. Moreover, it should be considered that even for a single enzyme reaction, the optimum temperature changes, depending upon the substrate concentration or incubation time. Also, the inactivation temperature varies, depending upon the particular enzyme.

Moisture content

The moisture content and circumstance in which enzymes are present has a profound effect on enzyme activity. In the presence of sufficient substrate, lack of moisture can inhibit enzyme reactions because all enzyme reactions occur in aqueous systems. There are soluble commercial enzymes which are stable in their dry powder state but start to react as soon as the enzymes are exposed to water.

2.2.3. Enzyme applications in food industry

Using enzymes in the baking industry

Amylases were the first enzymes to be added to bread dough. While initial use was for generating fermentable sugars, current interest centers on their ability to retard crumb firming, the anti-staling effect. During baking, between the gelatinization temperature and the enzyme denature temperature, amylases act on the damaged or gelatinized starch, producing dextrins,

oligosaccharides, and fermentable sugar. As a result, amylases in breadmaking increase gassing power and retard the crumb firming rate. There are three different sources of amylase used in breadmaking, cereal α-amylases, fungal α-amylases, and bacterial α-amylases. These α-amylases show different heat stabilities, which make differences in their functionality in breadmaking.

Proteases hydrolyze the peptide bond in proteins, being sometimes compared with reductants for their functionality in breadmaking. Exo-proteases increase the color and flavor of bread. Endo-proteases reduce the mixing time, fermentation time and elasticity. Especially, protease is used for mellowing strong flours. As a result, increased pan flow, finer grain, and softer texture may be obtained.

Endoxylanases with specificity towards water unextractable arabinoxylan (WU-AX) contribute to increased dough stability in two ways, by increasing the amount of WU-AX in dough on the one hand, and by increasing the amount of soluble arabinoxylan (AX) on the other hand (Figure 2.4). In addition to improvements in loaf volume, crumb structure, and crumb softness, the addition of optimal dosages of endoxylanases during breadmaking results in increased fermentation stability, resistance to mechanical stress, and greater oven rise. At higher enzyme dosages, slack doughs and dough stickiness after mixing become limiting parameters because of a loss in water holding capacity of the WU-AX.

Using enzymes in the milling industry

Enzyme supplementation is practiced both by addition to the flour at the mill and by the baker. It is well known fact that α-amylase preparations are added as a supplement to the flour at the end of milling. In addition to the addition of α-amylase in flour, enzyme application in milling have been studied to increase milling efficiency, i.e. improved quality of flour, higher product yield, and reduced milling steps, etc.

Previous studies (Haros et al 2002) reported that enzyme treated wheat, using such as cellulase, xylanase, and beta-glucanase during tempering, had a positive influence on improving the quality of the final products, especially bread, with respect to volume, crumb, and firming rate of bread. This enzyme pretreatment modified the initial structure of the wheat carbohydrates. The study suggested an alternative method to improve the final bread quality and overcome the enzyme distribution problem caused by nonuniform mixing and overdosage

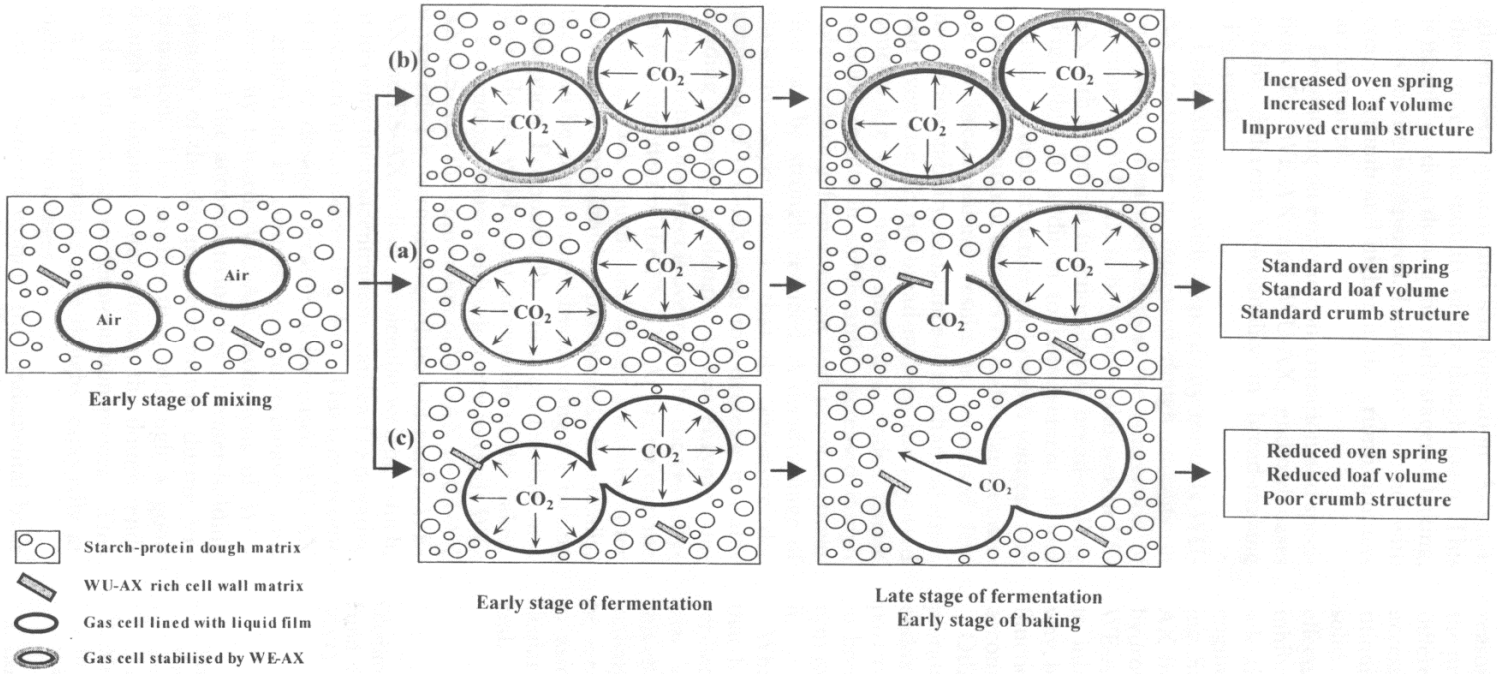


Figure 2.4 The mechanism behind endoxylanase functionality in breadmaking.

Model (a) represents the control situation with no endoxylanase added. There is little stabilization of liquid films by water extractable arabinoxylan (WE-AX) and a negative impact on gas cells of WU-AX. Model (b) represents the situation occurring when an endoxylanase with selectivity for WU-AX is added to the recipe. WU-AX are solubilized and the amount of WE-AX/ES-AX (enzymically solubilized arabinoxylan) increases. Coalescence between two gas cells is delayed. Model (c) represents the situation when an endoxylanase with selectivity for WE-AX is added to the recipe. WE-AX are extensively hydrolyzed, which results in decreased stabilization and increased coalescence of the gas cells compared to the control situation. The detrimental effect of WU-AX remains.

(adapted from Courtin and Delcour, 2002)

problems (slack and sticky dough) which occurred when enzymes were added directly to the flour or dough with other ingredients. However, the enzyme effect on bran separation from endosperm is unknown.

The use of commercial cellulases have been studied and well established. Hirao et al. (1963) studied starch recovery from cellulase enzyme pretreated cereals (rye, milo, corn, and barely) by the disintegration of the aleurone layer. Also, Takahaski et al. (1966) succeed in reducing the steeping time in corn wet milling by using cellulase. Al-Suaidy et al. (1973) studied the effect of cellulase treatment on wheat milling. It was considered that, as hemicellulase and cellulase hydrolyze the bran layer which is rich in cellulose and hemicellulose, the chemical composition of the bran layer might be modified. They could cause a change in the milling behavior and physical properties of the wheat kernel. From this study, it was found that, during cellulase treatment, the aleurone layer cells disintegrated as enzyme concentration increased. However, it was not enough to alter the milling properties. He suggested that it might be possible, through proper strain selection and better culture media, to produce a cellulase enzyme potent enough to cause such modification.

The effect of (1→4)-β-endo-xylanase treatment on wheat bran was studied (Benamrouche et al., 2002). By using UV fluorescence microscopy, this study confirmed the degradation of the aleurone cell wall after (1→4)-β-endo-xylanase treatment. After 24 h incubation, the aleurone layer was completely lost. However, the tissues in the outermost layer of the bran retained their integrity during xylanase treatment. They also reported that 80% and 51.8% of the total carbohydrate was liberated from the hydrolysis of aleurone and inner bran respectively, whereas no carbohydrate was released by (1→4)-β-endo-xylanase treatment. Compared to the xylose/arabinose (X/A) ratios of aleurone layer, inner bran, and outer bran, which were 2.07 ± 0.06, 2.93 ± 0.03, and 0.79 ± 0.01 respectively, it was concluded that the degree of arabinose substitution (X/A) is one important factor for the high resistance of these polymers to endoxylanase degradation. For the exogenous enzyme, ferulates were thought to play an important role in modifying the mechanical properties of cell walls as well as in limiting polysaccharide degradation, functioning as a barrier to the hydrolytic enzymes by acting as cross-links between polysaccharides and between polysaccharides and lignin.

Saxena et al. (1993) examined the effect of enzymatic pretreatment on pigeon pea grain milling, and after that Arora et al. (2007) observed the optimum process parameters for the

milling of enzymatically pretreated rice. To obtain a higher quality of finished product (polished white rice), three process parameters (enzyme concentration, incubation time, and incubation temperature) were examined and optimized for developing an efficient milling system. The data was analyzed according to response surface methodology (RSM), showing that with enzymatic pretreatment, the rice bran layer softened up, being removed easily in the mechanical polisher. Cellulase used in the study acted on the bran layer and cell wall, helping break down the bran layer and cell wall structure. A lipase activated along with the cellulase degraded the oily outer bran layer, which functioned as a barrier to water penetration, leading to a reduction in the cooking time.

Synergism

Enzymes have degree of specificity to their substrates, and through various studies it has been found that they often have synergistic effects when used together in a system which is a complex of different kinds of polymers. In 1998, Zheng and Bhatty applied an enzyme cocktail containing cellulase, endo-(1→3),(1→4)-β-D-glucanase and xylanase in the wet separation of starch, extracting a higher yield of starch from hullless barley, as compared with the conventional procedure. In 1991, Steinke and Johnson reported that incorporating multiple enzymes in steeping solutions reduced steeping time and enhanced starch separation during wet-milling of maize. Padmanabhan et al. (1993) reported a significant improvement in starch recovery from cassava by using pectinase and cellulase. Petit-Benvegnen et al. (1998) showed the synergistic enzyme effect of endoxylanase and ferulate esterase on cell wall degradation. As the endoxylanase was used with ferulate esterase, the elimination of some of the ferulic acids improved the accessibility and binding of endoxylanase on the arabinoxylans.

Hille and Schooneveld-Bergmans (2004) examined the synergistic effect of fungal and bacterial endoxylanases in breadmaking. The volume of final products with much less of the combination of these enzymes was similar to those with higher amounts of a single enzyme. Another type of synergy resulted from the addition of cellulase and/or cellobiohydrolyase to the fungal and bacterial endoxylanase. Cellulases open and break down the cellulose which is intertwined with the arabinoxylan polymer, helping the endoxylanase activity. This synergistic effect had a positive influence on bread volume and crumb softness as well.

2.3. Wheat Milling

2.3.1. Processes Affecting the Milling Efficiency

Every step, from planting the seeds to harvesting, and each step of the milling process, represent important factors affecting milling efficiency. Here, conditioning and pretreatments such as debranning, soaking, freezing, and drying of wheat will be considered.

Conditioning (tempering)

Optimum moisture contents for wheat milling vary widely, depending upon whether the wheat is hard or soft, with hard wheat generally conditioned to 15.5 to 17% moisture content and soft wheat to 14 to 15.5% moisture content. To obtain a high yield and high flour quality, tempering (resting) times also range from 12 to 24 hours, depending on the wheat variety and tempered wheat moisture content. Butcher and Stenvert (1973) defined the flour yield related to the resting time and tempered wheat moisture content for various kinds of wheat. After this study, they also reported that a peak in flour yield did not correspond to the moment when the moisture was completely distributed across the wheat kernel. To achieve optimum milling performance, a certain well defined radial moisture gradient across the grain was required, with the periphery being at a higher moisture level than the center of the grain (Butcher and Stenvert, 1973). Lee and Stenvert (1973) pointed out factors affecting the water penetration during the conditioning, such as hydrophilic pentosan content (arabinoxylans), thickness, and physical nature of the bran. They confirmed that the degree of branching (in this study, it was presumed that the ratio of arabinose/xylose reflect differences in branching) in the bran arabinoxylan is of considerable importance in determining the water penetration, in that the rate of water penetration of hard wheat was slower than that for soft wheat.

Pretreatments

There have been various efforts to increase milling efficiency by changing the physical or chemical properties of the wheat kernel before milling. Fisher and Hines (1939) stated that wetting and drying made the wheat swell and created cracks or enlarged preexisting cracks. Similarly, Grosh and Milner (1959) suggested that wetting created stresses due to the moisture difference between the wet and dry portions, which induced the cracks. This cracks served as a

water penetration pathway across the wheat kernel. In 1941, Swanson studied the effects of wetting and drying of mature wheat on the grinding and milling quality. As a result, he observed that kernel test weight and vitreousness were changed but that flour yield and baking quality were not changed. Watson et al. (1967) reported that wheat which underwent weather changes, repeated rain, snow and freezing, during the harvest season showed high milling and baking quality. Adams and Naber (1971) found water soaking improved the nutritive value of wheat for poultry. In 1973, Al-Suaidy et al. reported that softening hard wheat by soaking and drying may be economical, since milling hard wheat required more power and caused more roll repair and sieve replacements than did milling soft wheat (Greenaway, 1962).

From the several studies on the effects of bran on flour quality, it is evident that debranning might improve flour refining, increasing its milling yield in parallel. The Satake Company invented a flour milling pretreatment process which consists of 3 steps of polishing followed by adding water at the last step. They reported that through 3 polishing steps, the pericarp, seed coat, and aleurone layer cell walls were removed and the internal aleurone cell content would be washed out by adding water to raw wheat grains (U.S patent 5,846,591). Pandiella et al. (2004) studied the debranning effect on cereal food quality and reported that the use of debranning technology in flour milling could produce a nutritious flour including the aleurone part, or at the same time the isolated aleurone fraction could be used as a functional ingredient for the production of enhanced value cereal based foods.

Based on the literature review, it appears that the application of enzyme in temper water before wheat milling might have some beneficial effects. This study was planned to determine 1) the parameters which affect enzymatic reactions on probable enhancement of bran separation and 2) the effect of enzyme application in temper water on wheat milling. These parameters were optimized to develop a protocol for the application of enzymes in the temper water, and conditions during tempering. Also, the effects of enzymatic tempering on bran separation during the milling process were studied as compared with the conventional (control) process. A wheat bran fraction was first used to determine if the enzymes work on bran and, if so, to establish the appropriate conditions such as different enzyme concentrations, incubation temperature, and incubation time which enzymes were likely to enhance bran separation during milling if enzymes were used in the tempering water. After milling, test baking using the flour milled from the

enzymatic tempered wheat was conducted in order to see if the enzyme treatment in temper water had the effects on the bread making and bread staling.

CHAPTER 3 - Enzyme Activity on Wheat Bran

Prior to the application of enzymes to intact wheat kernels, enzymes were applied to wheat bran to observe if there was a hydrolyzing effect on wheat bran, and if so, what effects the enzymes have.

3.1 Materials and Methods

Enzymes

Cellulases, xylanases, and pectinases were obtained from Specialty Enzymes and Biochemicals Co. (CA, U.S.). According to the specification sheet provided by the supplier, each of the enzymes showed different optimum conditions for their activity (Table 3.1). Each enzyme and the combination of cellulase and xylanase were applied to wheat bran in the stipulated amount.

Table 3.1 Summary of Commercial Enzyme Characteristics Supplied by the Manufacturer[a]

Properties	Cellulase	Xylanase	Pectinase[b]
Appearance	Off white/ light tan powder	Light tan powder	Liquid
Solubility	Soluble in water	Soluble in water	Soluble in water
Activity	5000 CU/gm	5000 units/gm	2335 PGU/ml
Moisture	Not more than 0.7%	-	-
E.Coli	Negative	Negative	-
Salmonella	Negative	Negative	-
TPC	Less than 3000 CFU/gm	Less than 3000 CFU/gm	-
Opt. pH	5.5-6.0	4.0-6.5	3.5-6.0
Opt. Temp.	37-40 °C	55-60 °C	40-55 °C

[a] Specialty Enzymes and Biochemicals Co. (2006)
[b] In addition to the pectinase enzyme, it contains traces of cellulase, hemicellulase, and protease activities

Wheat bran

A blended hard red winter wheat from a local elevator near Manhattan, KS was milled in the KSU Grain Science and Industry department's in-house pilot flour mill. The resulting bran was obtained and stored in double plastic bags at 4°C until needed. The proximate analysis of wheat bran, on a dry basis, was: starch 19.7%, protein 20.9%, fibrous carbohydrate 45.4% (cellulose and hemicelluloses), lignin 3.5%, fat 3.2%, and ash 7.2%. This wheat bran was used to determine the enzyme activities and optimum conditions prior to application on wheat kernels.

Enzyme treatment of wheat bran

One hundred grams of wheat bran and 16 g of water with enzyme or water without enzyme were blended for 10 minutes in the tempering drum. Enzymes were added at stipulated concentrations (w/w = enzyme weight/tempering water weight). Treated wheat bran samples were transferred to a polyethylene sample bag and kept in an oven or under room conditions for the stipulated temperatures and times. Five g of wheat bran were then blended with 75mL of water, and centrifuged at 3,000 x g for 10 min at room temperature. The decantate was analyzed for carbohydrate reducing ends (monomer and polymer carbohydrates released by enzyme hydrolysis) with the dinitrosalicylic acid (DNS) procedure as described below. Enzyme treated bran was dried and used for scanning electro microscopy (SEM) (Model S-3500N, Hitachi Science Systems, Japan) to compare with the intact wheat bran.

Reducing sugar assay

The effect of enzymes on bran and their activities was related to reducing-end sugar determination for monomers and polysaccharides by the 3,5 dinitrosalicylic acid assay as described by Miller (1959), but volumetrically modified to a total of 1.5 mL reactant. Dextrose was used as a standard.

Experimental design

Response surface methodology is an incomplete block experimental design that fits a second order multiple regression equation to the data. The principle advantages are that it accounts for non-linear effects (most of nature is non-linear) and checks for interactions, and it can predict data for combinations not actually tested, provide three dimensional contour plots, and most importantly it can substantially reduce the number of experimental runs required. Once

the identified “vital few” controllable factors are decided upon, with suitable software or graphical means, it is even possible to find the ‘best’ combination for all variables in concert (Walker, 2002).

The experiment was conducted according to the requirement of response surface methodology (RSM) for analyzing data regarding the optimum conditions for enzyme activities. A second order Box-Behnken design was conducted with 3 levels, high, medium, and low, for 3 treatments factors, enzyme concentration, incubation time, and incubation temperature (Table 3.2 and 3.3). The results were analyzed and plotted by software, RSM Plus (AEW consulting, version date: 1992) using the model, STD 3_VAR.

Table 3.2 Response Surface Methodology (RSM) design

	x	y	z
1	+1	+1	0
2	+1	-1	0
3	-1	+1	0
4	-1	-1	0
5	+1	0	+1
6	+1	0	-1
7	-1	0	+1
8	-1	0	-1
9	0	+1	+1
10	0	+1	-1
11	0	-1	+1
12	0	-1	-1
13	0	0	0
14	0	0	0
15	0	0	0

Random order for center points. Replicates were fixed.

Table 3.3 Variables and levels

		-1	0	+1
x	enzyme concentration (%) (w/w)	0%	0.75%	1.5%
y	incubation Temp (°C).	25 C	45 C	60 C
z	incubation time (hr)	1 h	4 h	16 h

3.2 Results and Discussions

Reducing sugar assay results are shown in Figure 3.1, 3.2, 3.3, and 3.4 for cellulase, xylanase, pectinase, and the combination of cellulase and xylanase treatment, respectively. The data were analyzed and plotted by the software, RSM Plus. This graph is made of alphabetical letters; and each letter corresponds to the released reducing sugar concentration in the supernatant. These results show a hydrolysing effect of enzymes on wheat bran. The left side and right side graph presented the released reducing sugar concentration at 0 hour and 16 hours incubation time, respectively. The X-axis is enzyme concentration and Y-axis is incubation temperature.

The coefficients of determination (R^2) were 0.9925, 0.9168, 0.9640, and 0.9768 for cellulase, xylanase, pectinase, and the combination enzyme, which means that the model fits the data for enzyme activity very well for all three treatments, enzyme concentration, incubation time, and incubation temperature. From the results, as time and concentration of enzyme increased, the released sugar in the supernatant increased for the three enzymes and the combination of xylanase and cellulase. Within the range of experimental treatments, cellulase, pectinase, and the combination of cellulase and xylanase all show higher activities at the higher temperature and enzyme concentration for longer incubation time. However, although we expected that xylanase would be more active at high temperature, the predicted response surface for the released reducing sugar concentration by xylanase appeared to be a bowl shape with a minimum sugar release at about 0.677% enzyme and 40 °C; and the highest released reducing sugar concentration was observed at two different temperature ranges, high and room temperature, when the same amounts of enzyme were applied on the bran.

Equations (1), (2), (3), and (4) shown below were obtained from the RSM Plus program for the activities of cellulase, xylanase, pectinase, and the combination of xylanase and cellulase respectively, to predict the released sugar concentration. These equations indicated the relation between the individual treatments, and interactions between treatments and released sugar concentration.

Equation (1) for cellulase:

Released sugar, $S = 11.4158376 – 1.4923129E – 0.2211565T + 0.7071543t – 0.0608220ET – 0.0019356Tt + 0.0738555Et + 2.8650230E^2 + 0.0033280T^2 – 0.0189655t^2$

Where E = enzyme concentration (%),

t = incubation time (hr), and

T= incubation temperature (°C)

Equation (2) for xylanase:

Released sugar, $S = 5.0668739 + 0.4954408E + 0.0860984T + 0.3080767t + 0.0202577ET + 0.0082901Tt – 0.0176452Et –0.3651399E^2 – 0.0012739T^2 – 0.0222804t^2$

Equation (3) for pectinase:

Released sugar, $S = 8.3926584 + 0.1926668E + 0.0285524T + 0.0049771t + 0.0037312ET + 0.0076526Tt + 0.0050051Et + 0.1996072E^2 - 0.0002917T^2 – 0.0112482t^2$

Equation (4) for the combination of xylanase and cellulase:

Released sugar, $S = 7.9475436 + 1.4723567E – 0.0917968T + 0.1359924t – 0.0335219ET + 0.0130425Tt + 0.0310216Et + 0.7490712E^2 + 0.0013247T^2 – 0.0227563t^2$

It was found that enzyme concentration and incubation time had the most effect on the released sugar concentration for the cellulase, xylanase, and their enzyme cocktail activities. Unlike cellulase and xylanase, when pectinase was used, the incubation temperature effected the released sugar concentrations slightly more than the incubation time did. When the same amounts of enzyme (1.5% w/w) were used for the bran hydrolysis, the amount of released sugar measured 13.76 mmol for the cellulase, 15.94 mmol for the xylanase, 13.97mmol for the pectinase, and 16.67mmol for the combination of xylanase and cellulase. The combination of two enzymes, cellulase and xylanase, showed a synergistic effect on hydrolyzing the bran fraction slightly, which implies a possible synergistic effect of an enzyme cocktail including all three enzymes, xylanase, cellulase, and pectinase.

Scanning electro microscopy (SEM) showed an interesting picture of the bran fraction (Figure 3.5). The intact bran layer was covered with starch granules and aleurone cell walls.

However, after enzyme treatment, the starch granules might have been washed off and the inner aleurone cell walls were revealed. The aleurone cell contents were released due to the rupture of aleurone cell walls.

It was confirmed that the carbohydrases, xylanase, cellulase, and pectinase, had an effect on hydrolyzing the bran layers, resulting in releasing some of the aleurone cell content. Although higher enzyme concentrations, longer incubation times, and higher incubation temperatures helped the enzyme to act on the bran layer, the usage level of enzyme, incubation time, and temperature should be considered in terms of the economic aspect. It is suggested that the use of an enzyme cocktail consisting of cellulase, xylanase, and pectinase, combined for their synergistic effect, be considered. Also, other conditions influencing enzyme activity which were not tested in this study, such as pH and substrate moisture content, should be examined to optimize the effect of enzyme activities.

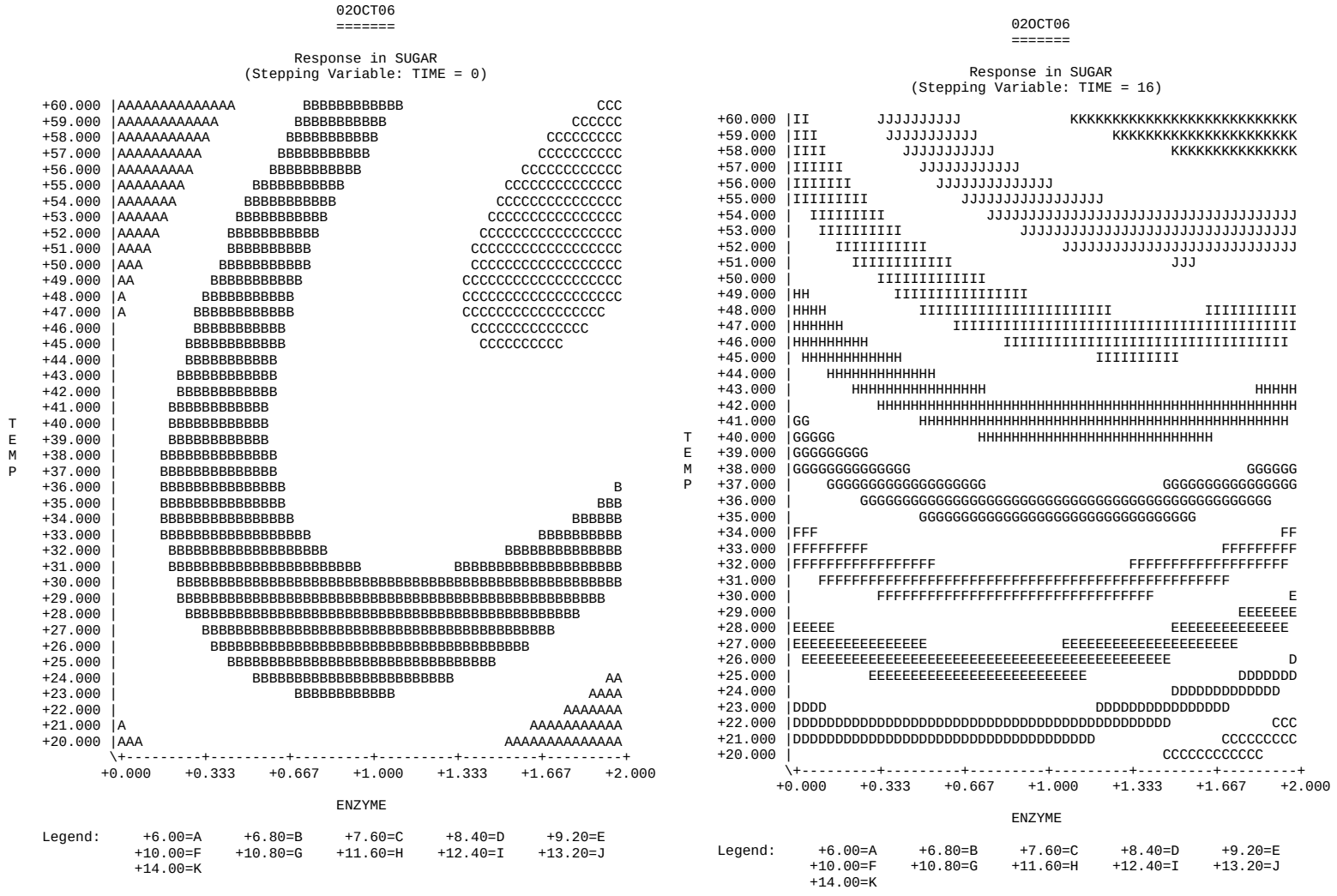
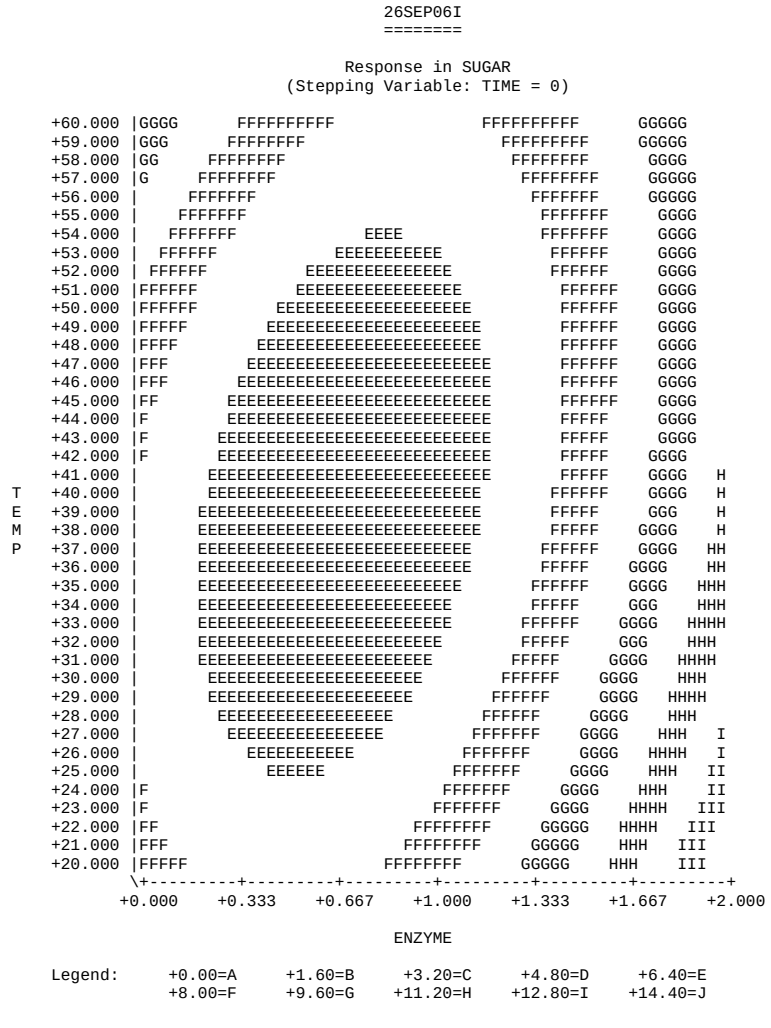


Figure 3.1 RSM plots for cellulase activity. 0 hour incubation (left) and 16 hours incubation (right)



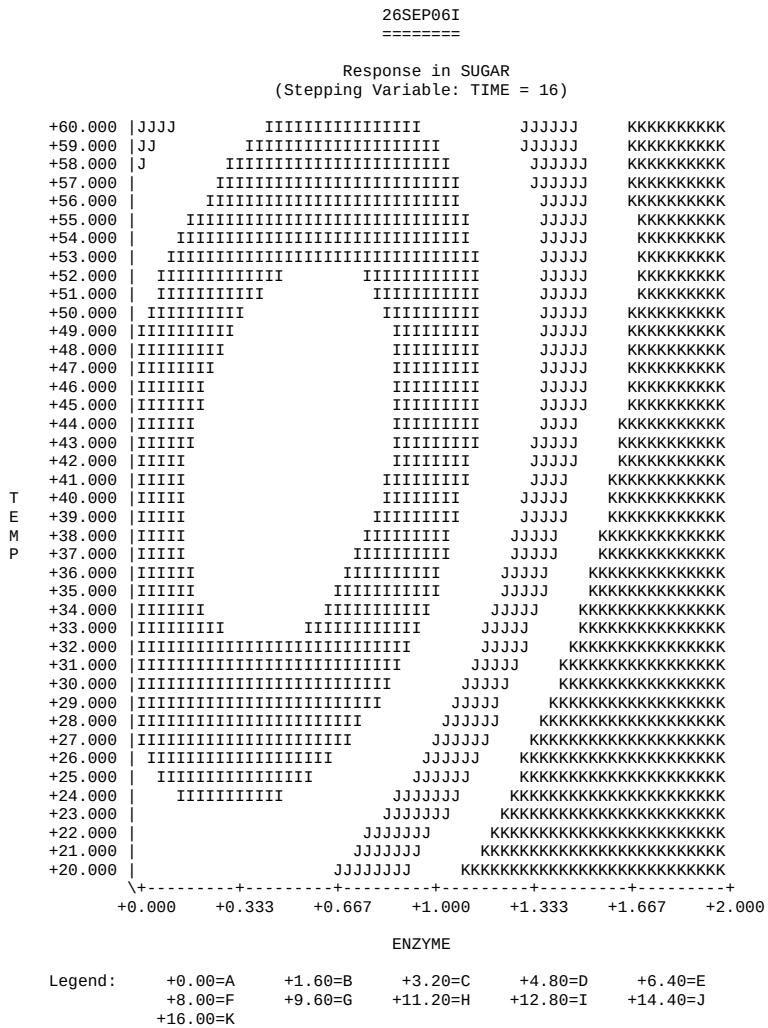


Figure 3.2 RSM plots for xylanase. 0 hour incubation (left) and 16 hours incubation (right)

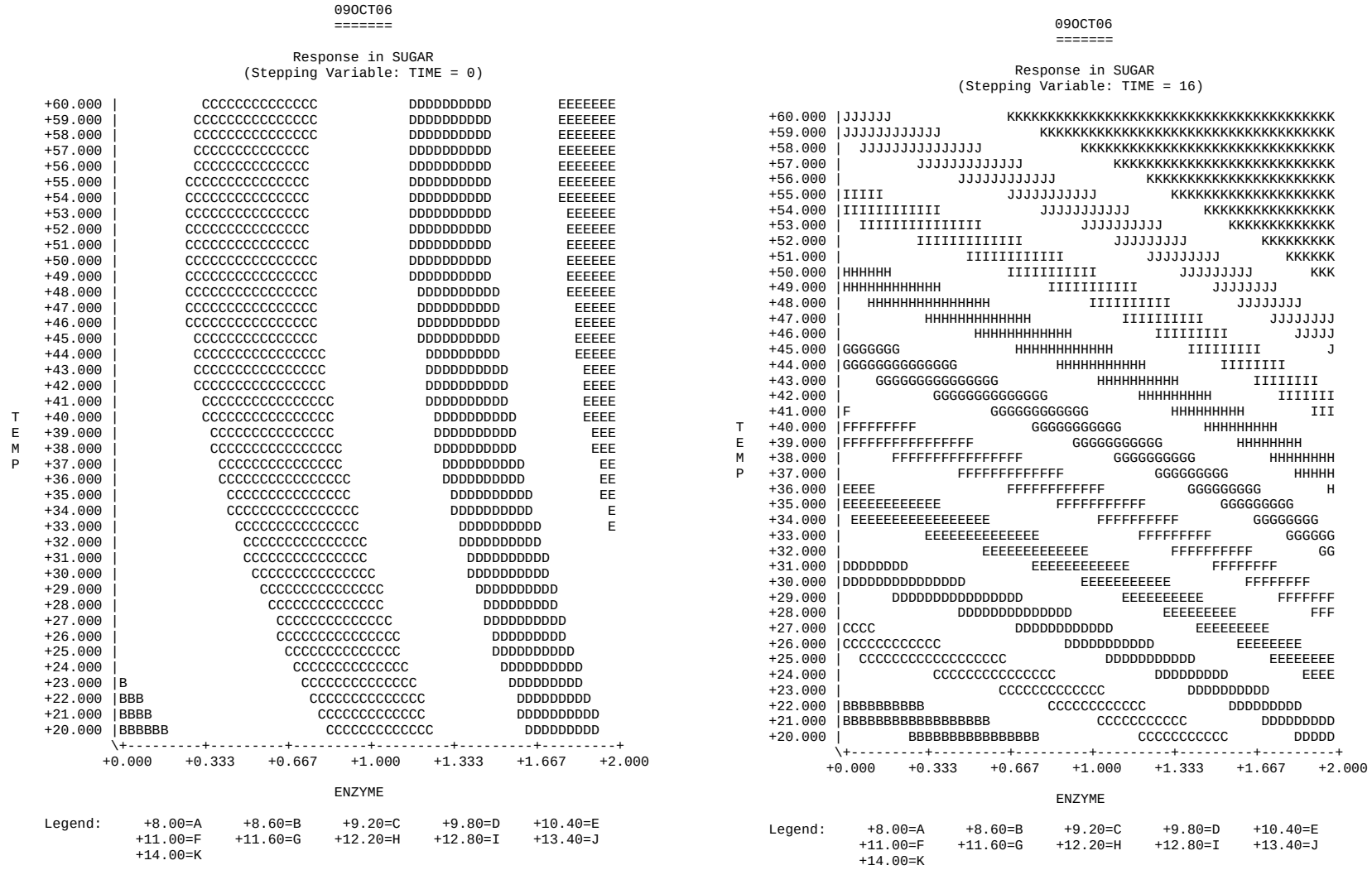


Figure 3.3 RSM plots for pectinase activity. 0 hour incubation (left) and 16 hours incubation (right)

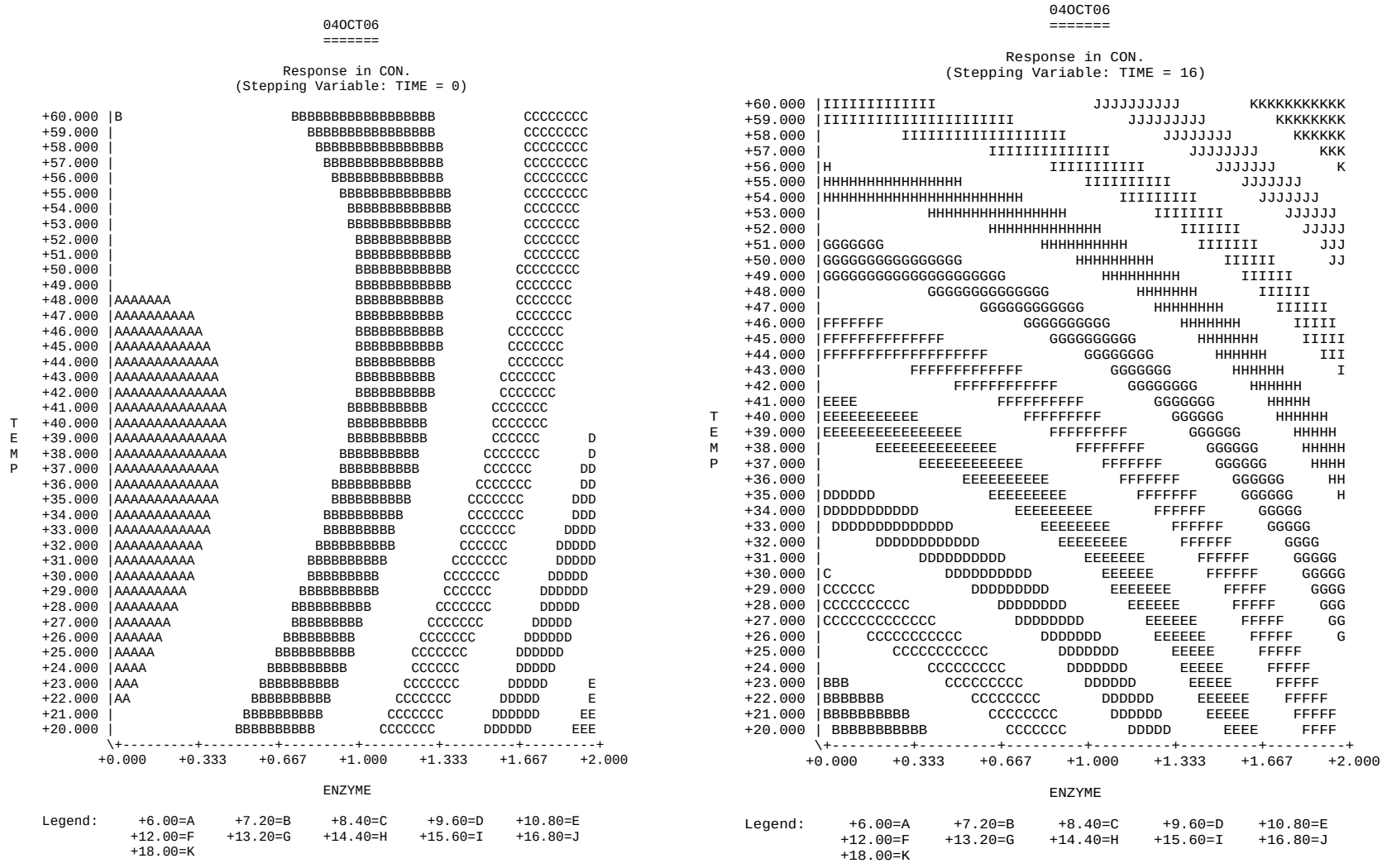


Figure 3.4 RSM plots for combination of xylanase and cellulase. 0 hour incubation (left) and 16 hours incubation (right)

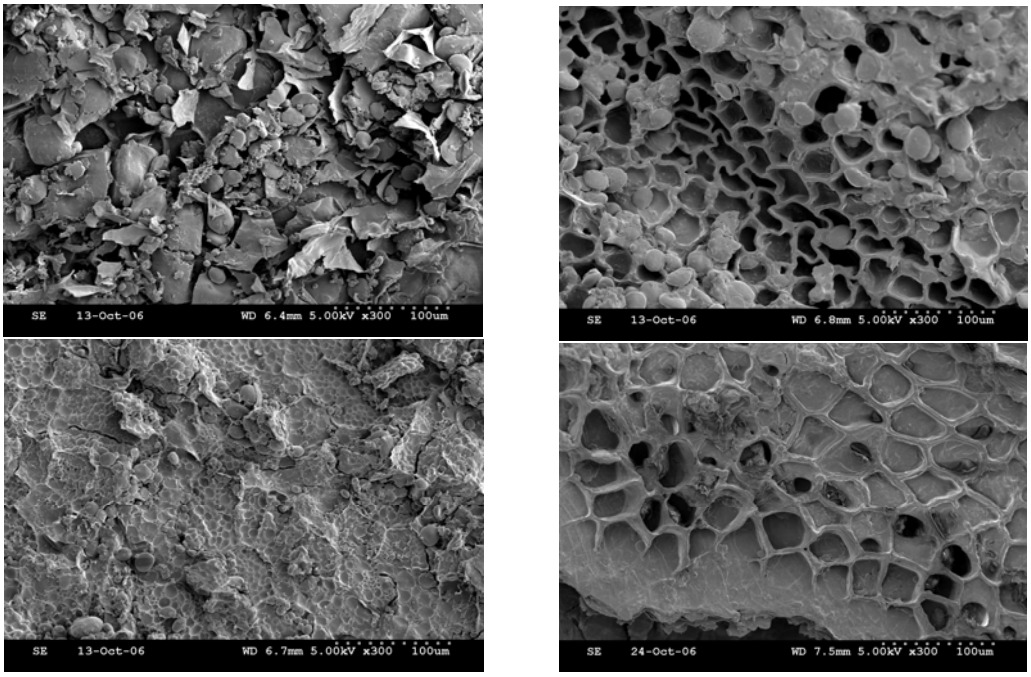


Figure 3.5 Scanning electron microscope (SEM) pictures of inner bran fraction. Intact bran fraction (left) and enzymatically hydrolyzed bran (right). Intact bran fraction is covered with aleurone cell walls whereas the enzymatically hydrolyzed bran fraction exposes the inner aleurone cell wall and aleurone content was released.

CHAPTER 4 - Bran Separation on Enzyme Treated Wheat

Based on results from the preliminary test with wheat bran, an experiment for the enzyme application to intact wheat kernels was designed.

4.1 Materials and Methods

Destarched bran

Prior to running the reducing sugar assay for determining the enzyme activities, destarched bran was prepared as a substrate because, as mentioned previously, the wheat bran contained 19.7% starch. To remove starch from wheat bran and obtain a pure wheat bran fraction, 1 g of α-amylase, SEB® AMYL-XCP (*Aspergillus oryzae*, Specialty Enzymes Co.), and 150 g of wheat bran were incubated at optimum temperature (45°C) for 2 hours in 1200 mL of pH 5 water. The wheat bran was then dried at 65°C, and the α-amylase was inactivated at that temperature.

Enzymes

Cellulases, xylanases, and pectinases were donated by Specialty Enzymes and Biochemicals Co. (CA, U.S.). An enzyme cocktail, comprising a combination of xylanase, cellulase, and pectinase, was prepared in equal ratios based on their activities. These amounts of enzymes were found to produce one locally-defined 'sugar unit' from 1 g of destarched bran substrate when incubated for 2 hours at optimum temperature and pH (40°C, pH 6 for cellulase, 55°C, pH 5 for xylanase, and 45°C, pH 5 for pectinase). After enzyme application on the destarched wheat bran, the mixture was centrifuged at 10,000 x g for 10 min at room temperature, and then the reducing sugar assay was conducted with the supernatant. One unit is defined as the amount of enzyme which can hydrolyze the substrate and releases 15 mmol of reducing sugar in the supernatant in 2 hours incubation time. As a result, 1 unit of the enzymes, cellulase, xylanase, and pectinase, was definded as 31.68 μg, 26.36 μg, and 127.94 μL, respectively (Figure 4.1).

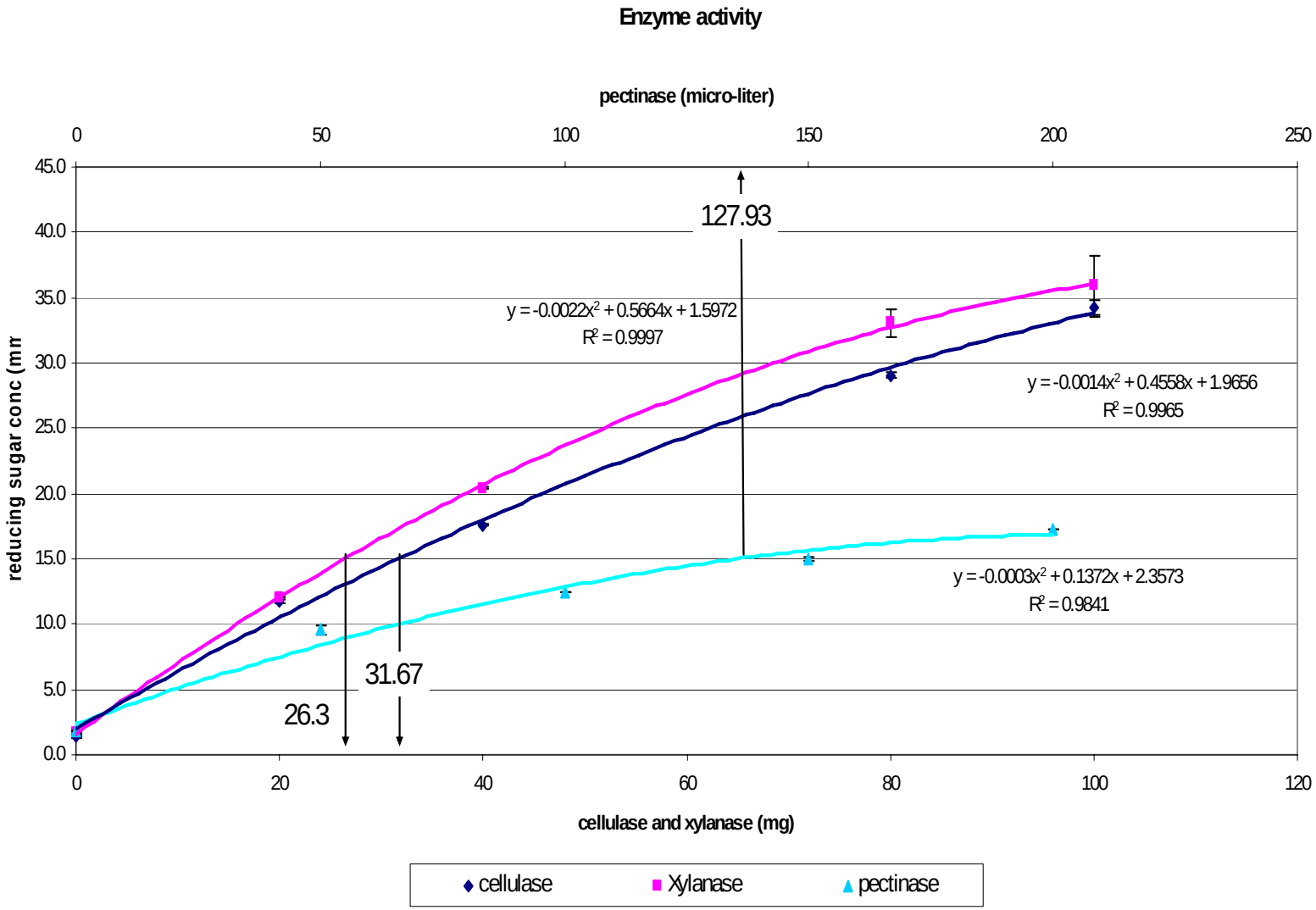


Figure 4.1 Enzyme activities for cellulase, xylanase, and pectinase by reducing sugar assay

Wheat kernel

A pure variety sample of hard red winter wheat (2174) was procured from the Agronomy Department, Kansas State University. Test weight was 60.3 lb/Bu. Single kernel weight, diameter, hardness, and moisture were measured by using the Single Kernel Characterization System (SKCS) model 4100 (Perten Instruments North America, Inc., Reno, NV) , and were 33.94 mg, 2.72 mm, 63.78 (hardness index), and 12.02%, respectively. By using the oven drying method (AACC 44-15A), moisture content of the wheat was 12.36%; and the required amount of water was determined following the tempering table (AACC 26-95).

Wheat preparation and milling process

Five hundred grams of wheat were tempered to the postulated amount of moisture content following the experimental design (Table 4.4). The required amount of water and enzymes were calculated and added to the wheat. Distilled water was mixed with 37% hydrochloric acid to adjust pH to 3, 4, 5, or 6. For pH 7, distilled water was used for tempering. The enzymes were dissolved completely before adding to the wheat. Wheat samples were shaken with water or enzyme solution in a 2-layer sealed plastic bag for 3 minutes, and then incubated for a stipulated time and temperature in the oven. After the prescribed incubation times, wheat which was tempered above 16%, was spread out on the sieves as a single layer, and dried back to 16% moisture content in a 32°C oven. Prior to milling, the physical properties of the kernel were measured by using the single kernel characterization system (SKCS 4100). For grinding, the experimental laboratory Ross Mill flow, which consisted of four breaks, one sizing, two tailings, and five reductions, was used. The experimental milling flow is shown in Figure 4.2. The amount of flour from each of 13 streams, bran, shorts, red dog, and germ was recorded on the experimental mill data sheet (Appendix A) for later analysis.

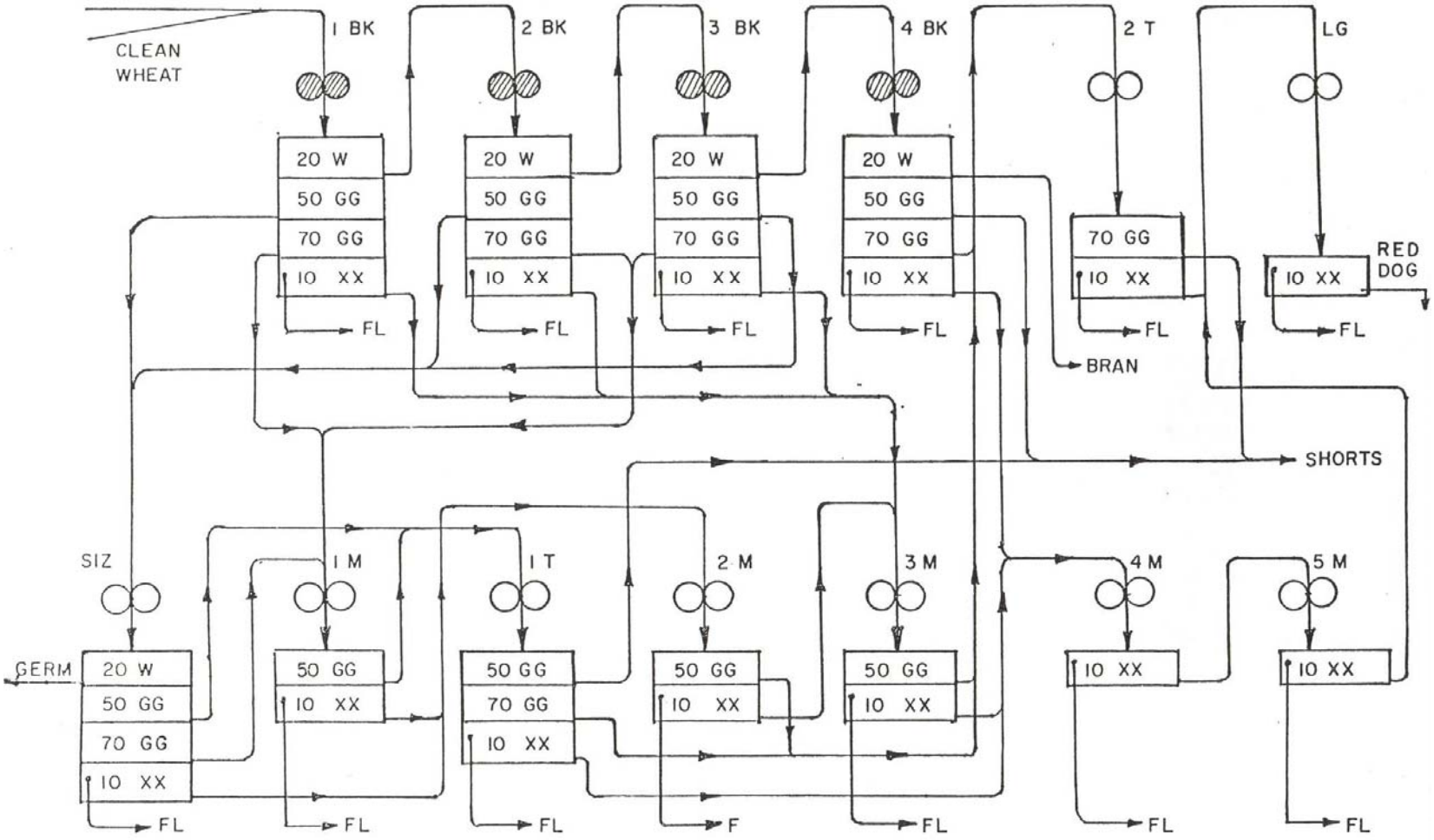


Figure 4.2 Experimental Milling Flow sheet for Ross Rolls Batch Procedure

(adapted from Posner et al. 1997)

BK: Break, SIZ: sizing, T: Tailing, M: Middling, LG: Low grade, and FL: Flour

Quality Parameters for Flour and Milling Process

The flour obtained from each grinding was classified into patent and clear flours and then analyzed for flour quality and milling efficiency. To determine the quality and efficiency of the milling process, quality parameters, namely flour yield, flour protein and ash content, flour particle size, and flour color were studied. Protein and ash were measured following AACC 46-30 and AACC 08-01 respectively. For the flour color, the Agtron green light reflectance color meter (Model M-45-D, Agtron Inc. NV) was used. Gillis (1963) reported that the Agtron green light could be used to determine the flour ash content and was more closely related to changes in ash than was the Blue Agtron light. Also, it has been found that the Agtron green light reading was stable during 6 months of storage under ambient condition (Shuey, 1975).

Experimental design

The experiment was conducted according to the requirement of response surface methodology (RSM) for analyzing the data regarding the milling efficiency and flour quality. Central composite designs are economical in terms of experimental units; and enable the estimation of quadratic response equations (Kuehl, 2000). A second order central composite design was conducted with 5 levels, high, medium high, medium, medium low, and low, which were coded by -2, -1, 0, +1, and +2 respectively, with 5 variables, enzyme concentration, incubation time, incubation temperature, tempering water pH, and tempering target moisture content (Table 4.1 and 4.2). The result was analyzed and plotted by SAS (version 9.1, SAS Institute, Inc., Cary, NC, USA) at $p<0.05$. Response surface analysis was used to estimate the model coefficient and to perform a response surface regression (RSREG) procedure by SAS. The "ridge min" and "ridge max" options in the RSREG procedure were included to generate the ridge of maximum and minimum response of the dependent variables. The total required number of experiments was 33 for the each treatment, and the levels and required experiment number was balanced (Table 4.3 and 4.4). Actual experimental order was randomized.

Table 4.1 Variables and Levels

			-2	-1	0	+1	+2
X1	Enzyme concentration	Units	0	60	120	180	240
		% (w/w)*	0	0.84	1.70	2.55	3.40
X2	Incubation time	hr	6	9	12	15	18
X3	Incubation temperature	°C	25	32.5	40	47.5	55
X4	Target moisture content	%	16	18	20	22	24
X5	Tempering water pH	pH	3	4	5	6	7

* Added enzyme concentration based on the dry matter of the wheat kernels

Table 4.2 Balanced Number of Required Experiments

Levels	X1	X2	X3	X4	X5
-2	1	1	1	1	1
-1	8	8	8	8	8
0	15	15	15	15	15
+1	8	8	8	8	8
+2	1	1	1	1	1
Total runs	33	33	33	33	33

Table 4.3 Response Surface Methodology for Central Composite Design with 5 Variables

X1	X2	X3	X4	X5
-1	-1	-1	-1	1
1	-1	-1	-1	-1
-1	1	-1	-1	-1
1	1	-1	-1	1
-1	-1	1	-1	-1
1	-1	1	-1	1
-1	1	1	-1	1
1	1	1	-1	-1
-1	-1	-1	1	-1
1	-1	-1	1	1
-1	1	-1	1	1
1	1	-1	1	-1
-1	-1	1	1	1
1	-1	1	1	-1
-1	1	1	1	-1
1	1	1	1	1
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0
-2	0	0	0	0
2	0	0	0	0
0	-2	0	0	0
0	2	0	0	0
0	0	-2	0	0
0	0	2	0	0
0	0	0	-2	0
0	0	0	2	0
0	0	0	0	-2
0	0	0	0	2
0	0	0	0	0

: 7 replicates

Table 4.4 Randomized Experimental Design Run Order

run	Enzyme Con. X1	Incubation time X2	Incubation Temp X3	Tempered MC X4	Tempering water pH X5
1	120	12	40	0.2	7
2	60	9	47.5	0.22	6
3	120	12	40	0.2	5
4	120	12	25	0.2	5
5	180	15	32.5	0.18	6
6	60	15	47.5	0.18	6
7	60	9	32.5	0.18	6
8	120	6	40	0.2	5
9	120	12	40	0.16	5
10	180	9	32.5	0.22	6
11	120	12	40	0.2	5
12	180	15	47.5	0.18	4
13	120	12	40	0.2	5
14	60	15	47.5	0.22	4
15	60	15	32.5	0.18	4
16	120	18	40	0.2	5
17	0	12	40	0.2	5
18	120	12	40	0.2	5
19	120	12	40	0.2	5
20	120	12	40	0.2	5
21	180	15	32.5	0.22	4
22	180	9	32.5	0.18	4
23	180	9	47.5	0.18	6
24	120	12	40	0.2	3
25	240	12	40	0.2	5
26	180	15	47.5	0.22	6
27	60	9	47.5	0.18	4
28	120	12	40	0.24	5
29	60	9	32.5	0.22	4
30	60	15	32.5	0.22	6
31	120	12	40	0.2	5
32	120	12	55	0.2	5
33	180	9	47.5	0.22	4

: 7 Replicates

4.2 Results and Discussions

4.2.1 Effect on Flour Color

The results that were measured by the Agtron and Minolta were analyzed by SAS software (Appendix D) and the contribution of the independent variables to the response surface for color was investigated. Agtron readings for the various treatments ranged from 72 to 79, 51 to 68, and 70 to 77 for patent, clear, and straight flour, respectively (refer to Appendix C). For both tests (Agtron and Minolta) there were no significant factors affecting the flour color ($p<0.05$); and the stationary point was at a saddle point. Because the canonical analysis resulted in a saddle point, the estimated surface did not have a unique optimum. Further analysis, ridge analysis with radius 2.0, was required to see the color change pattern for the variables within the given range. In ridge analysis, it was observed that the flour Agtron color index increased slightly as the amount of enzyme and the pH were decreased, and as the incubation time was increased.

4.2.2 Effect on tempered wheat kernel physical characteristics

Significant factors affecting the tempered wheat kernel physical characteristics are summarized in Table 4.5. Time, interactions between time and moisture and between enzyme and pH were the significant factors affecting the single kernel weight after tempering, and resulting RSM equation explained 85.76% of variation in termed kernel weight. Incubation time was the only significant factor affecting the kernel hardness after tempering, and RSM equation accounting for 74.19% of variation in tempered kernel hardness was obtained. There was no significant factor for the tempered wheat diameter, and 73.41% of variation was explained by RSM equation. Only slight changes in physical characteristics were apparently caused by the 5 variables, as observed for the 33 data sets. Since the predicted response surface was a saddle shape, ridge analysis was conducted. For the wheat kernel hardness, the data indicated that kernel hardness increased from 62.47 to 64.10 as the enzyme increased from 120.00 to 120.03 units, incubation time increased from 12 to 12.18 hrs, incubation temperature increased from 40.00 to 40.10 °C, tempering moisture decreased from 20.00 to 18.74%, and tempering pH increased from 5.0 to 5.8.

4.2.3 Effect on the protein and ash content in flour

Significant factors and R-square for flour protein and ash content are shown in Table 4.5. The treatments were not statistically significant for the patent and clear flour ash contents with R^2 values, 0.43 and 0.54 respectively. Patent flour ash content ranged from 0.382 to 0.532; clear flour ash content ranged from 0.569 to 1.010 for the treatments (refer to Appendix C). For both flours, clear and patent, the result of ridge analysis of the predicted response surface showed that the ash content increased as pH decreased, with a slight change associated with the other treatments. Both the linear and the quadratic terms for tempering moisture content were the significant factors affecting the patent flour protein, whereas incubation time and the interaction between enzyme concentration and pH affected clear flour protein content. Patent flour protein content ranged from 10.37 to 10.92, and clear flour protein content ranged from 10.07 to 12.30 across treatments. Resulting RSM equation explained 79.38% and 61.91% of variation in patent and clear flour protein content, respectively. The correlations between flour ash content and Agtron color reading for patent flour, clear flour, and straight flour are shown in Figure 4.3, 4.4, and 4.5. For all three flours, inverse relations were found between ash and Agtron reading; but only the clear flours showed high correlations between ash content and flour color, with -0.76 for the correlation coefficient.

4.2.4 Effect on the Product Yield

Product yield for the various treatments was shown in Appendix C. The amounts of flour produced from the 13 streams were analyzed individually and then combined as patent, 1st clear, 2nd clear, and straight flour, and analyzed by SAS to observe the effect of treatments on wheat bran separation (Table 4.6). The stationary point for all predicted response surfaces were at the saddle point, which did not show an optimum condition. Most of the flours from break, sizing, and tailing streams were affected by the treatments, whereas none of the flours from the reduction roll were affected by the treatments. The break flours were mostly affected by the treatment. Enzyme by itself, and enzyme interactions with any other factors such as temperature, incubation time, and pH, showed the effect on yield of 1BK, 2BK, 1T, Bran, and 1st Clear flour production. Interactions among incubation time, temperature, moisture content, and pH had a significant influence on the response surface of the break flour yield. The significant factors affecting the response surface of each flour yield are summarized on Table 4.5. From the ridge

analysis, pH change was most obvious as a cause of the production yield change. For the production of shorts, red dog, and germ, all factors but enzyme showed their effect on decreasing or increasing their yield. The yield of 1st clear was well explained by the predictable response surface model with R^2=0.9134. Ridge analysis showed that 1st clear flour yield increased from 6.2% to 6.6% when enzyme, time, temperature, moisture, and pH varied from 120.000000 to 120.000109 units, from 12.000000 to 12.745712 hrs, from 40.000000 to 39.887734°C, from 20.000000 to 18.473474%, and from 5.000000 to 3.950702, respectively (refer to Appendix D). Like the 1st clear flour, the pattern of paten flour yield within the range of variables was estimated by ridge analysis and was found to increase with an increase in enzyme, time, and temperature, and a decrease in moisture and pH. Predicted product yield was determined by the model with 21 terms. It was simplified and expressed the product yield with a high R square value (R^2>0.75) so that the product yield could be predicted for any treatment conditions, which will be discussed later.

Table 4.5 Analyzed significant factors (at $p<0.05$) to the response surface of physical characteristics of tempered wheat and flour protein and ash content

	Significant factors	R-square
Kernel weight	b, b*d, a*e	0.8576
Kernel diameter	-	0.7341
Kernel hardness	b	0.7419
Clear flour ash	-	0.5438
Patent flour ash	-	0.4314
Clear flour protein	b, a*e	0.7938
Patent flour protein	d, d*d	0.6191
St. Flour color	-	0.7104

a: enzyme concentration

b: incubation time

c: incubation temperature

d: tempering moisture content

e: tempering water pH

*: interaction between two treatments

Table 4.6 Analyzed significant factors (at $p<0.05$) to the response surface of product yield

Yield of milling product	Significant factors	R-square
1BK	e*a, e*e	0.8580
2BK	a, a*c, b*c, c*d, a*e	0.8221
3BK	c*e	0.6461
4BK	-	0.5468
SIZ	c	0.7015
1M	-	0.5558
1T	a*c	0.7100
2M	-	0.6247
3M	-	0.5710
2T	-	0.6810
4M	-	0.6484
5M	-	0.7497
LG	-	0.5767
Shorts, red dog, and germ	b, d, b*b, b*c, d*d, c*e	0.8706
Bran	e*a, e*c	0.7617
Patent flour	b, d*c	0.7880
1^{st} clear flour	a, b, a*b, a*e, c*e, d*e, e*e	0.9134
2^{nd} clear flour	-	0.6125
Straight flour	b, a*a, a*c, b*d	0.8658

a: enzyme concentration

b: incubation time

c: incubation temperature

d: tempering moisture content

e: tempering water pH

*: interaction between two treatments

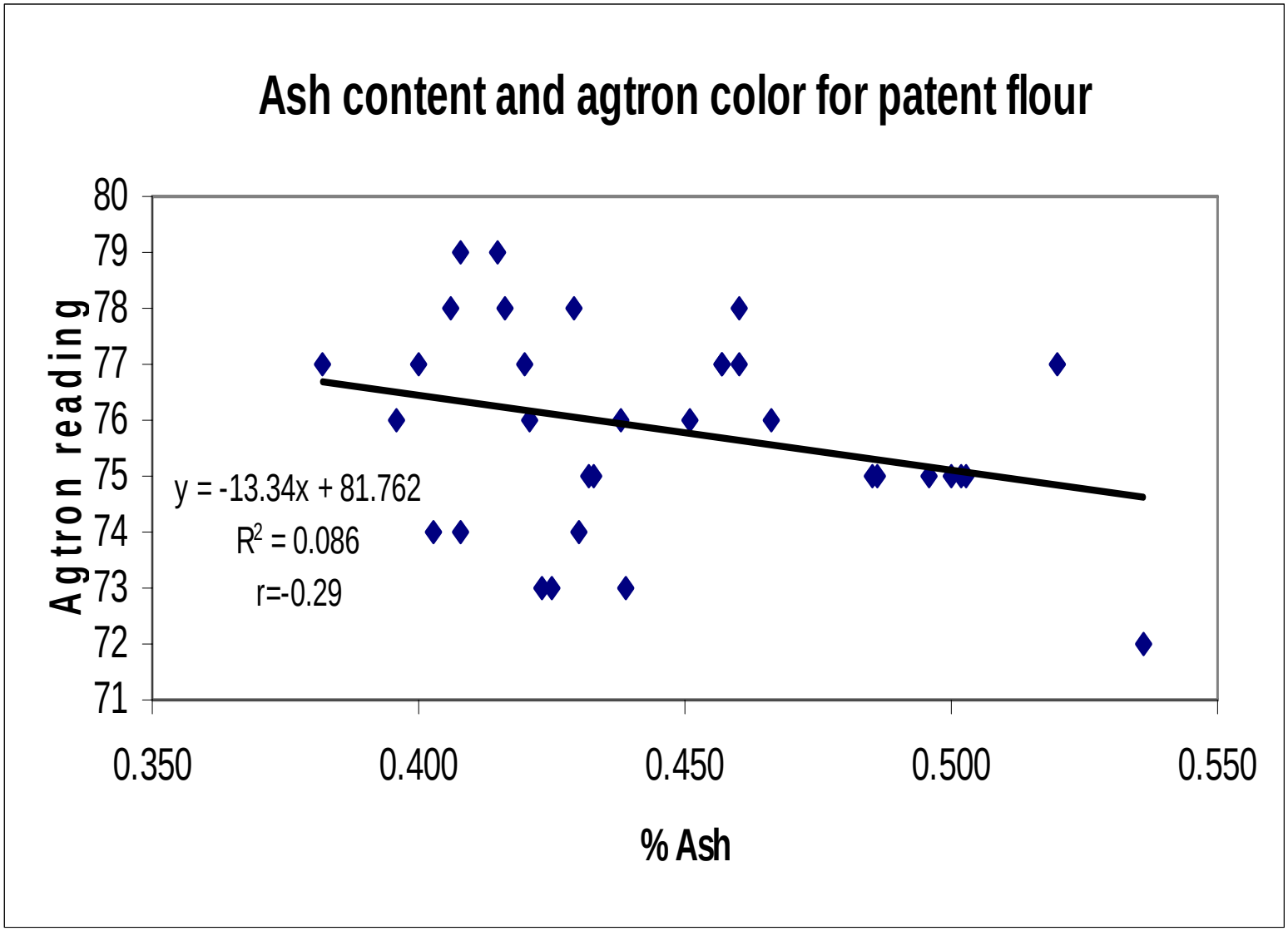


Figure 4.3 Scatter diagram of percent patent flour ash contents vs. Agtron reflectance values

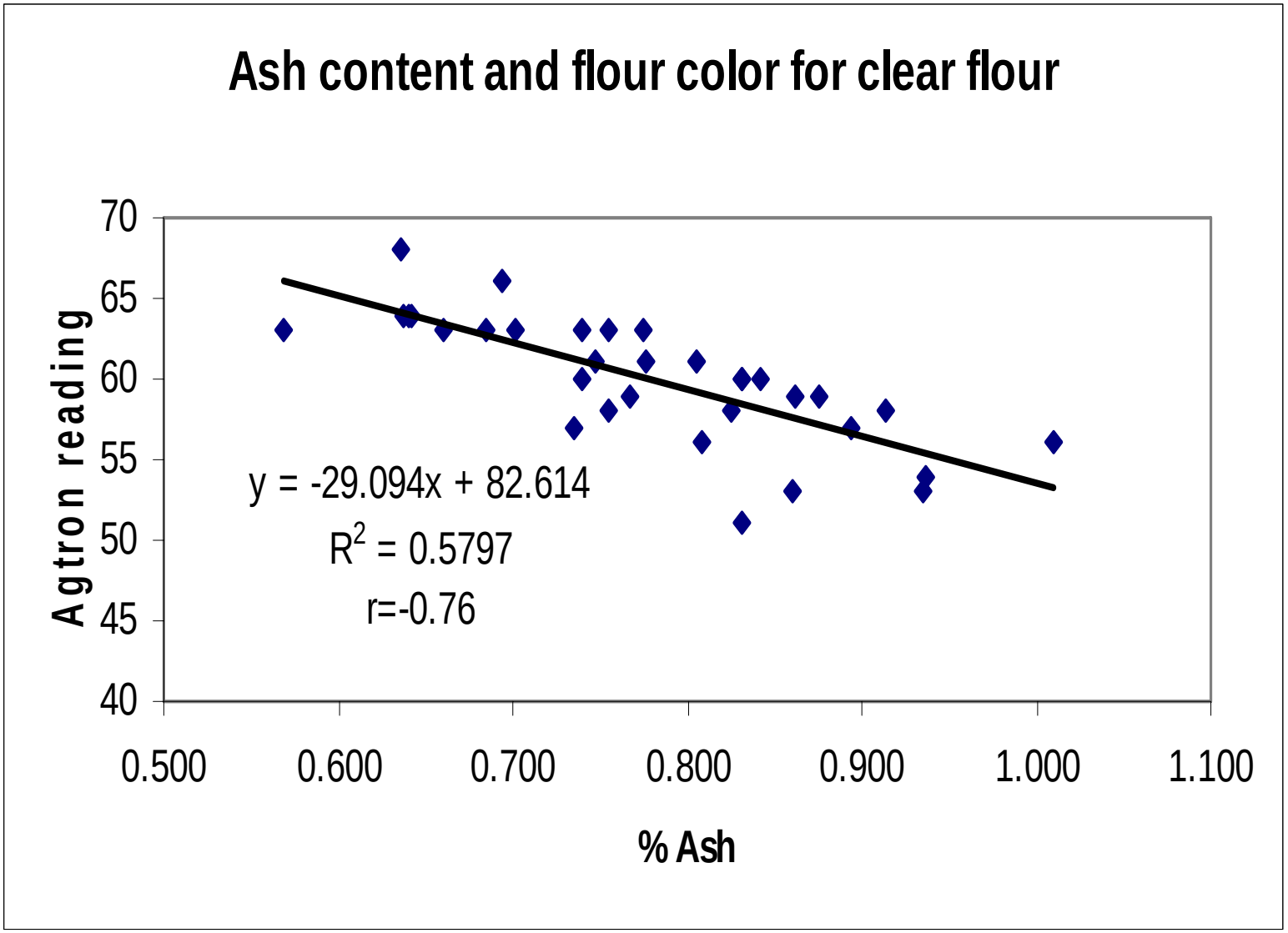


Figure 4.4 Scatter diagram of percent clear flour ash contents vs. Agtron reflectance values

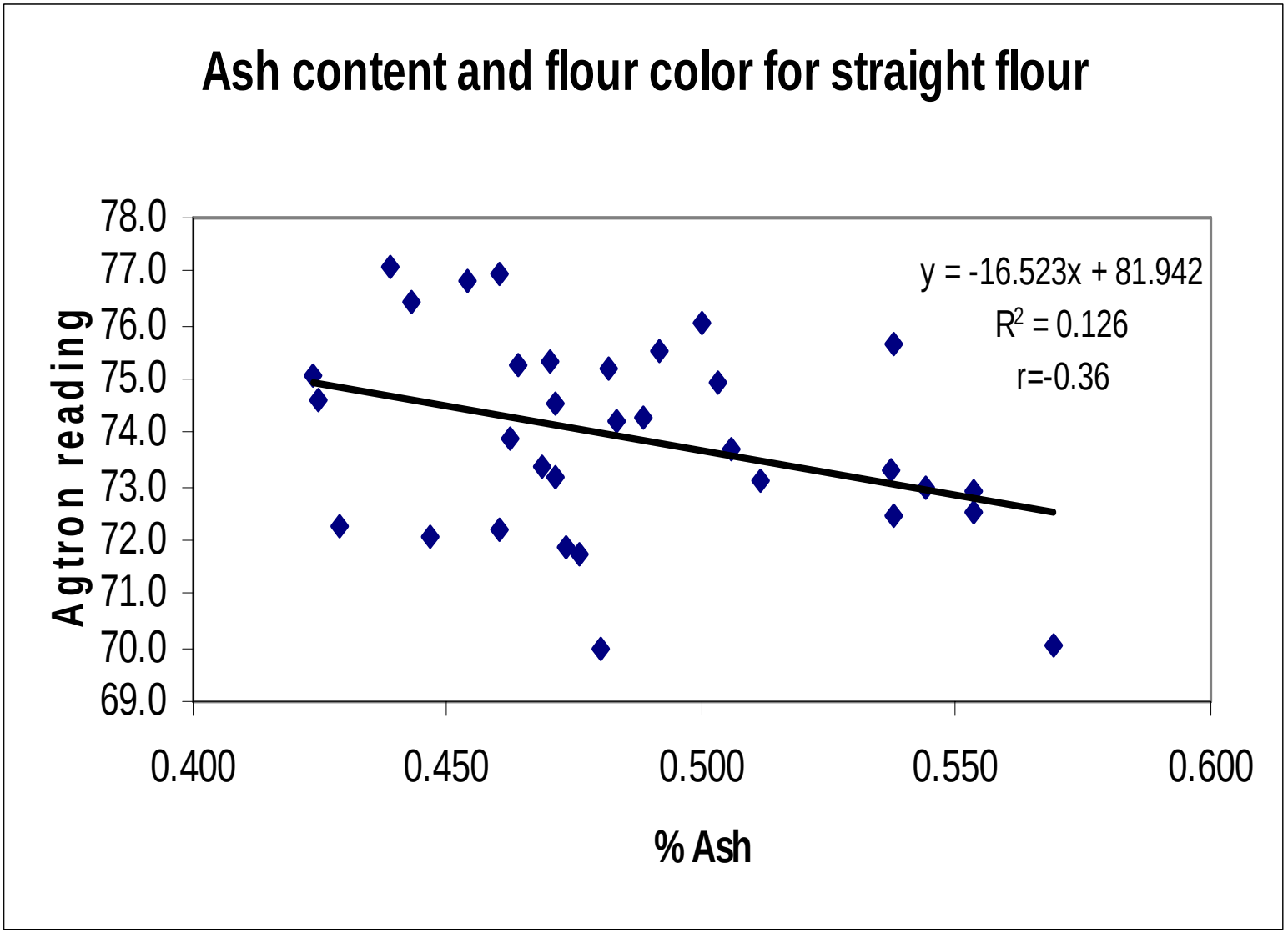


Figure 4.5 Scatter diagram of percent straight grade flour ash contents vs. Agtron reflectance values

4.2.5 Prediction of product yield

From the stepwise regression analysis using SAS software (Appendix D), quadratic models which show the relationship between the product yield and treatment variables were determined based upon 33 data sets. By stepwise regression analysis, quadratic models which can account for more than 75% of the variation in the experimental data, product yield, for the treatment variables were obtained. The selected model consisted of linear terms, quadratic terms, and cross-product terms for five treatment factors. The data for yield of flour from 1BK, 2BK, 1st clear flour, straight flour, patent, and the sum of shorts, red, and germ fit the model reasonably good ($R^2>0.75$); however, the yield data for the others did not fit as well ($R^2<0.75$) (Table 4.6).

The regression model fitted to the experimental results for the yield of 1st break flour showed a good R-square (0.8580). The simplified model after the R-square selection method showed $R^2=0.7513$:

Yield of 1st break flour = $6.66121 - 0.02296E - 0.11778P^2 + 0.00522EP - 0.00191tT + 0.01768tP$

Where E = enzyme concentration (unit), t = incubation time (hr), T = incubation temperature (°C), M = tempering target moisture content (%), and P = tempering water pH.

The regression model for the 2nd break flour showed a good R-square (0.8221). The model after the R-square selection method was obtained with $R^2=0.8161$:

Yield of 2nd break flour = $9.96279 - 0.03395E - 0.26139M + 0.00018786T^2 - 0.12447P^2 + 0.00028056ET + 0.00517EP - 0.00181tT + 0.00434tM + 0.00041742TM - 0.01191TP + 0.03810MP$

The model for the yield of 1st clear flour was expressed with $R^2=0.9134$ and obtained with $R^2=0.7773$ after the R-square selection method:

Yield of 1st clear flour = $6.76803 - 0.02379E + 0.00035171T^2 - 0.00436M^2 - 0.12324P^2 + 0.00008433Et + 0.00518EP - 0.01050TP + 0.03459MP$

The regression model for the yield of patent flour was expressed with R^2=0.7880 and obtained with R^2=0.7181 after square selection method:

Yield of patent flour = 11.48775 – 0.38040t – 0.38105M – 0.00002769E^2 – 0.00016582T^2 – 0.00073985M^2 – 0.13437P^2 – 0.00008204ET + 0.00268EP – 0.00007337tT + 0.01979tM + 0.03421MP

The regression model for the yield of straight flour was expressed with R^2=0.8658 and obtained with R^2=0.7655 after square selection method:

Yield of straight flour = 94.11942 – 2.10556t – 1.10560M – 0.00009795E^2 + 0.00095001ET – 0.00057835EM + 0.10208tM – 0.01708TP

The regression model for the yield of red dog, germ, and shorts was expressed with R^2=0.8706 and obtained with R^2=7515 after square selection method:

Yield of red dog, germ, and shorts = –59.72917 + 2.55949t + 0.85390T + 4.37230M – 0.06507t^2 – 0.13391M^2 + 0.00310Et + 0.00114ET – 0.00349EM – 0.03333tT – 0.11353TP + 0.24158MP

CHAPTER 5 - Enzyme Effects on Dough Properties and Bread Making

Dough property tests and baking tests were planned, and the flour for these tests was supposed to be selected based upon the flour protein contents for each of the treatments from the previous experiment. However, the protein and ash contents in flours did not appear to show any differences for the treatments; and it seemed difficult to compare the enzyme effects on the dough and baking quality since there were five treatments (enzyme concentration, incubation time, incubation temperature, tempering moisture, and tempering water pH). To compare the enzymes' effects on dough characteristics and bread making, it was necessary to simplify the experimental design in terms of the number of treatments. Only two factors, incubation temperature and tempering water pH, were controlled and samples compared with the control.

5.1 Materials and Methods

Hard red winter wheat

The same single variety of hard red winter wheat (2174) which was used for the previous experiment was used.

Wheat preparation and tempering

Fifteen hundred grams of wheat were tempered to 16% moisture content under 6 different tempering conditions (Table 5.1).

Table 5.1 Tempering condition

	Enzyme Concentration*	Incubation temperature	Tempering water
1 (control)	-	Room temperature	Tap water
2	3%	50°C	pH 5
3	3%	Room temperature	Tap water
4	-	50°C	pH 5
5	-	50°C	Tap water
6	-	Room temperature	pH 5

* w/w% based on the wheat kernel dry matter

Distilled water was mixed with sufficient 37% hydrochloric acid to adjust the pH to 5.0. The cocktail enzyme consisting of xylanase, cellulase, and pectinase with equal ratios of enzyme activity was dissolved completely in tempering water (3% w/w based on the dry matter of wheat) and then added to the wheat in a plastic container. The wheat was shaken in the plastic container for 3 min for uniform water distribution; and then 16 hours were allowed for rest or incubation time. An Isotemp® oven (Model 655F, Fisher Scientific Inc.) was used for 50°C incubation. After tempering, the tempered wheat was characterized by the SKCS.

Milling process

For the grinding, an experimental laboratory Ross Mill procedure consisting of four breaks, one sizing, two tailings, and five reductions, was used (procedure described above in Chapter 4). Product yield, and protein and moisture contents of the flour were determined.

Flour and dough characterization

Protein and moisture methods were based on AACC Approved Methods (AACC 2000) 46-30 and 44-15A, respectively. For the dough test, traditional empirical rheological methods such as the Mixograph (10 g flour bowl, National Manufacturing Division of TMCO, Lincoln, NE) and the Farinograph (50 g flour bowl, Farinograph E, C. W. Brabender, Duisburg, Germany), were conducted prior to the baking test to determine the optimum % water absorption and mixing times following AACC 54-40A and AACC 54-21, respectively.

Test baking

Test loaves (100 g flour) were baked following AACC Approved Method (AACC 2000) 10-10B. The formula was as follows: flour, 14% mb (100%), instant yeast (2.7%), sugar (6%), salt (1.5%), shortening (3%), malt flour (0.2%), and ascorbic acid (50 ppm). Fermentation temperature was 84-88°F, and the relative humidity was 95% in the fermentation cabinet. Optimum water absorption and mixing time was estimated by mixograph and corrected after mixing time pre-test. Mixing time pre-test was conducted with 100 g flour and shortening. Proofing height and baked bread weight and volume were obtained. The loaf volume was measured by rapeseed displacement method (volume meter). For the staling experiment, the Voland Stevens LFRA Texture Analyser (Voland Corp. Hawthrone, NY) was used to measure the staling rate for the treatments, using a 1- inch thick bread slice. Each treatment was baked

twice. Three slices were measured from each loaf, and the average for the 6 slices was taken for comparison purposes. The bread was stored in two layers of plastic bags at room temperature for one, three, and five days. The data obtained from the test baking were analyzed with ANOVA using SAS software at $p<0.05$.

5.2 Results and Discussions

Physical kernel characteristics

As shown in Figure 5.1, 5.2, 5.3, and 5.4, the results from the SKCS showed obvious effects of enzyme treatment on single kernel weight, diameter, and hardness. Kernels treated with enzymes had significantly higher weight, larger diameter, and greater hardness, irrespective of heat and pH treatments, compared with non-enzyme treated kernels. The increases in weight and diameter of the tempered kernels were not explained solely by their moisture contents. Small but significant differences in the weight of the kernel might be explained for by the weight of the enzyme applied. Three % (w/w) of enzyme was applied, based on the kernel dry matter, during the tempering, and it might account for the difference. For the kernel hardness, enzyme treated kernels were harder than non enzyme treated kernels and were harder than the kernel before tempering, which was against the usual purpose of tempering. Increase in hardness after enzymatic tempering was unexpected result and was in disagreement with well known fact that the larger kernel is softer. Interestingly, although enzymatic tempered wheat kernel was harder, it seemed to be more swollen, as compared with non-enzyme treated kernel.

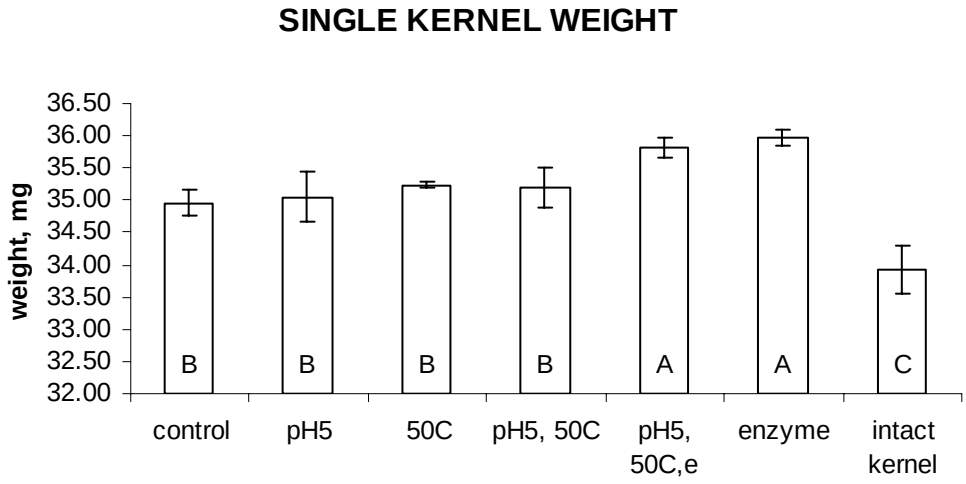


Figure 5.1 Single kernel weight after different tempering conditions

Letters indicate statistical differences at $p<0.05$ (n=2). The weight of the enzyme treated kernels was significantly higher than that of non-enzyme treated kernels.

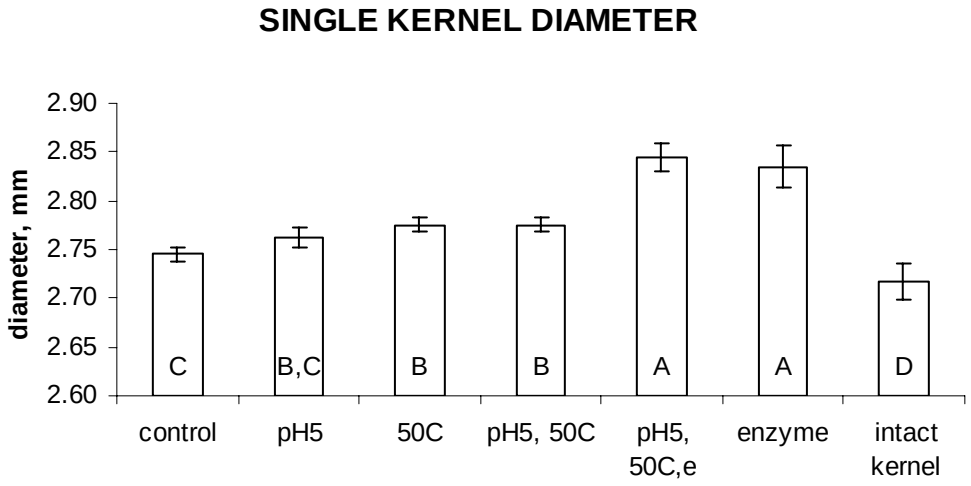


Figure 5.2 Single kernel diameter after different tempering conditions

Letters indicate statistical differences at $p<0.05$ (n=2). The diameter of the enzyme treated kernels was significantly higher than that of non-enzyme treated kernels.

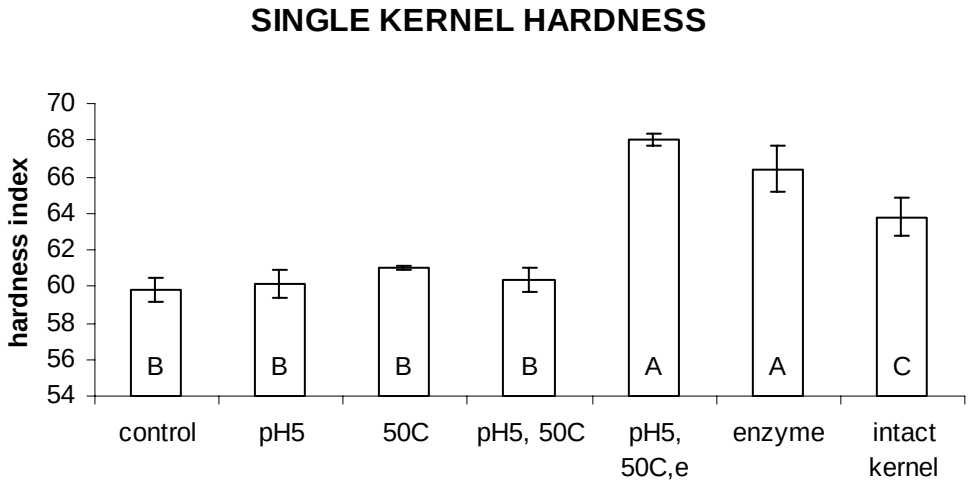


Figure 5.3 Single kernel hardness after different tempering conditions

Letters indicate statistical differences at $p<0.05$ (n=2). The hardness of enzyme treated kernels was significantly higher than that of non-enzyme treated kernels. After enzyme treatment with or without heat and under pH treatment, the kernel hardness was higher than that of intact kernels.

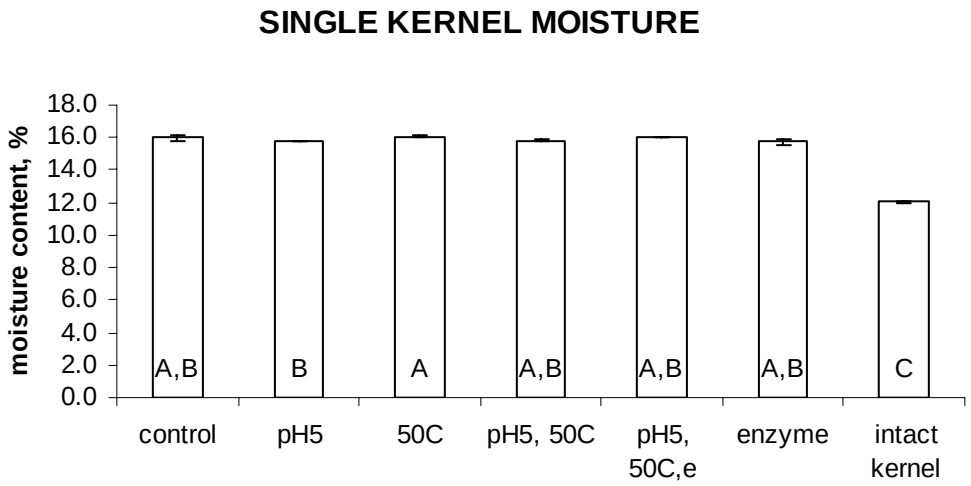


Figure 5.4 Single kernel moisture content for different conditions

Letters indicate statistical differences at $p<0.05$ (n=2). Moisture of tempered wheat was not significantly different for the treatments. The wheat kernels with 12.02% moisture content (by SKCS) were tempered to 16% moisture content.

Flour yield

Product yields for the patent, clear, straight flour, bran, red dog, germ, and shorts were not significantly different for the treatments, which corresponded to the previous results from the Chapter 4.

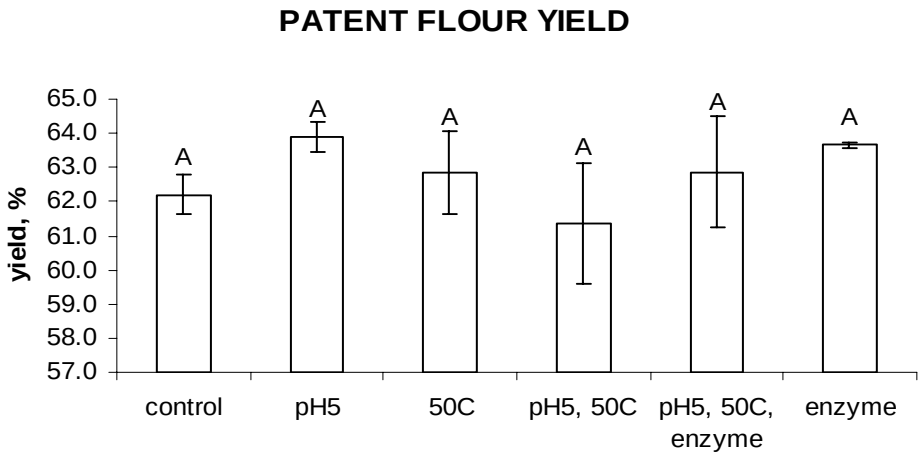


Figure 5.5 Patent flour yield for the treatments

Letters indicate statistical differences at $p<0.05$ (n=2). Patent flour yield for the treatments was not significantly different.

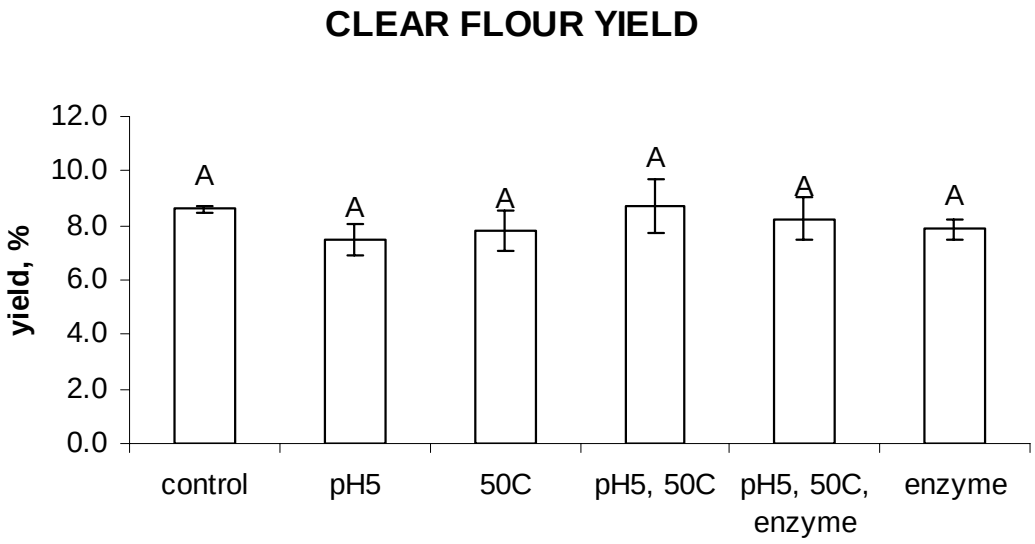


Figure 5.6 Clear flour yield for the treatments

Letters indicate statistical differences at $p<0.05$ (n=2). Clear flour yield for the treatments was not significantly different.

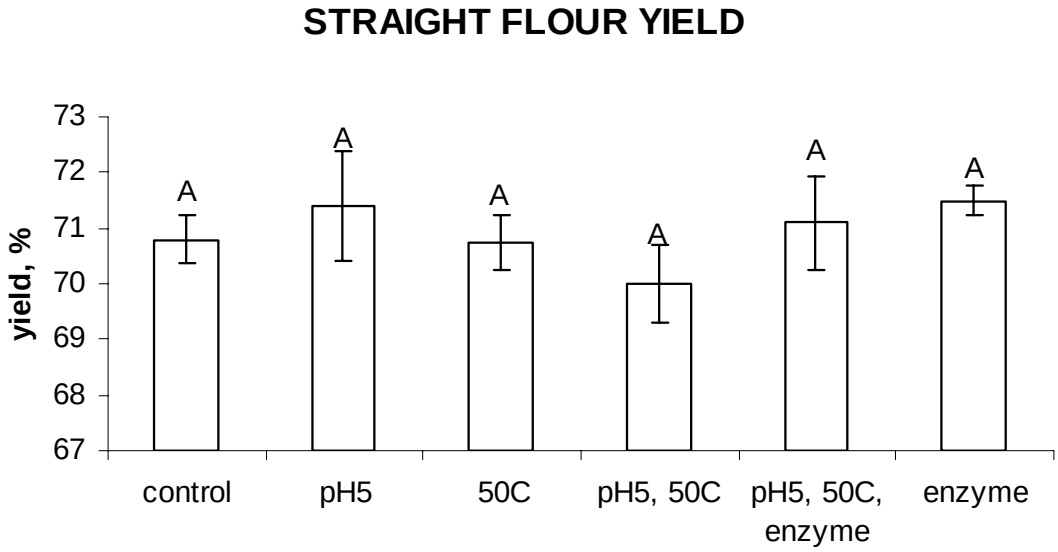


Figure 5.7 Straight flour yield for the treatments

Letters indicate statistical differences at $p<0.05$ (n=2). Straight flour yield for the treatments was not significantly different.

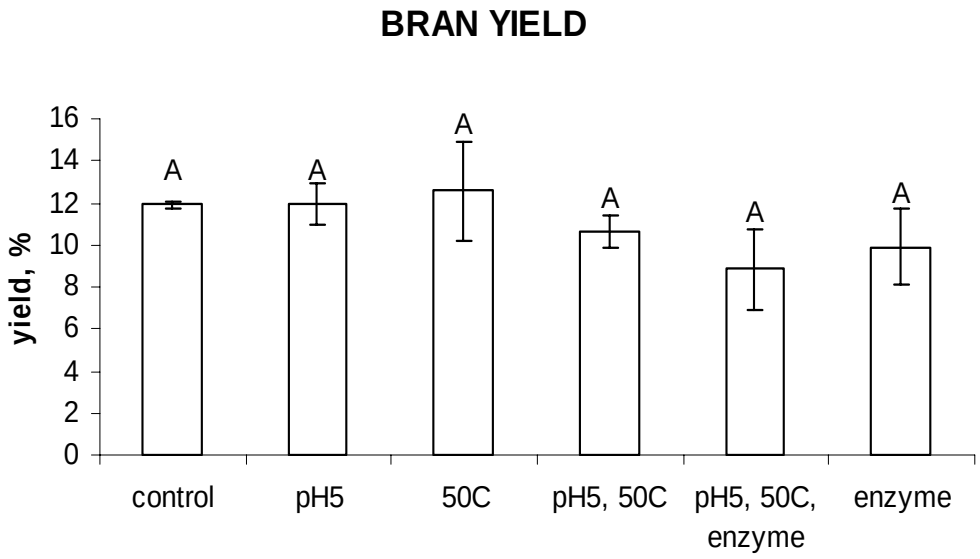


Figure 5.8 Bran yield for the treatments

Letters indicate statistical differences at $p<0.05$ (n=2). Bran yield for the treatments was not significantly different.

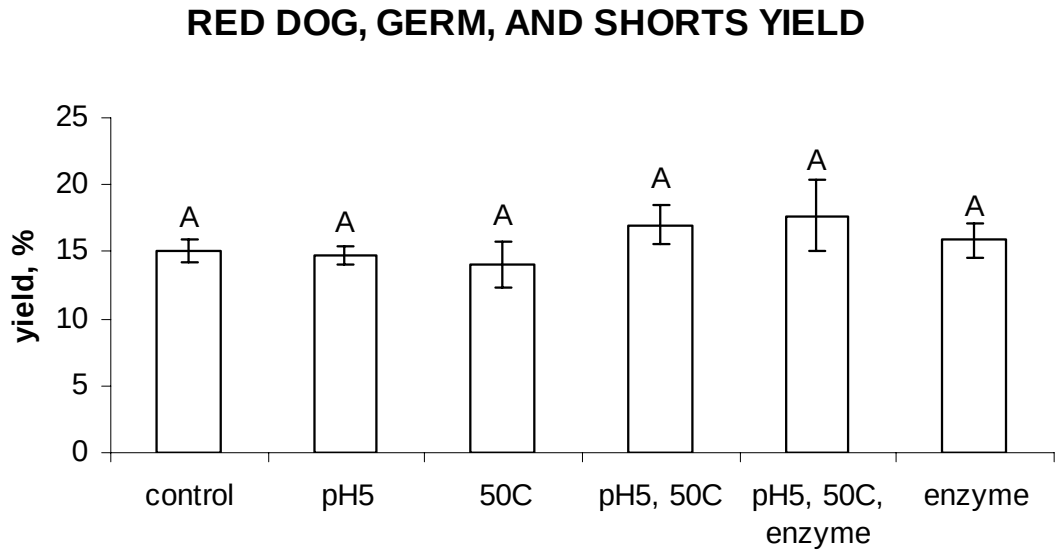


Figure 5.9 Red dog, germ, and shorts yield for the treatments

Letters indicate statistical differences at $p<0.05$ (n=2). Yield of red dog, germ, and shorts for the treatments was not significantly different.

Flour protein content

The protein contents of the flours for the treatments are shown in Figure 5.10. Significantly higher protein contents of the flours resulted from the enzymatically tempered wheat. Compared with the control, flour protein content was increased 2.18 and 2.94% for the enzyme treatment with heat under the pH treatment and only enzyme treatment, respectively. However, it could not be concluded that the increased protein is functional protein for the bread making because the mixograms did not show any differences in optimum water absorption or mixing time (described later), and also, there were no significant differences in clear flour yields. The applied enzyme amount (3% w/w based on dry matter of wheat kernels) could account for the increase in the protein level of flour from the enzyme treated kernel.

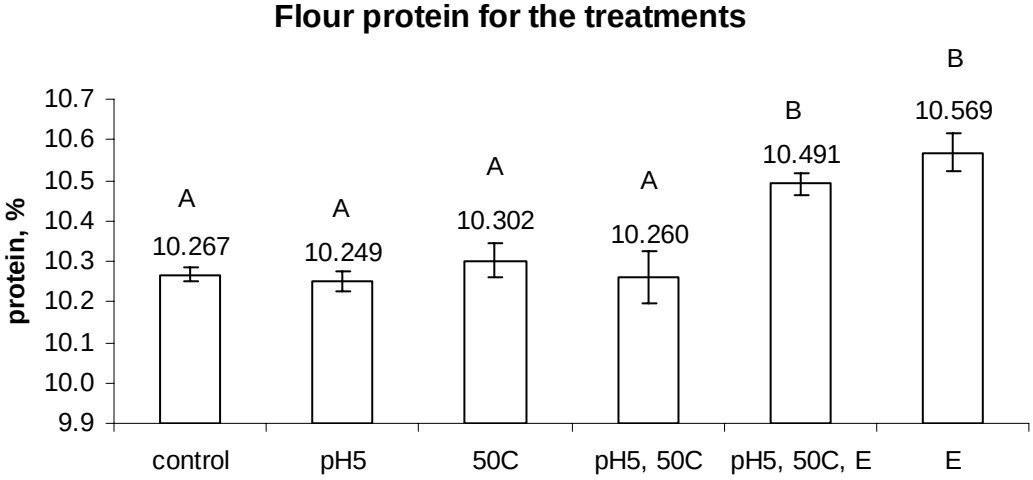


Figure 5.10 Flour protein content for the treatments

Letters indicate statistical differences at $p<0.05$ (n=2). Protein content in flour containing enzyme treatment was significantly higher, regardless of heat and pH treatment.

Farinograph

Estimates for the absorption ranged from 56 % to 57 %. Control flour and pH treated wheat flour had longer mixing stability. Heat-only treated and heat and pH treated wheat flour showed slightly shorter mixing stability than control and pH-only treated wheat flour. The flour milled from the enzymatic tempered wheat seemed to require slightly higher absorption and broke down more rapidly after the peak time had been reached (Figure 5.11).

Mixograph

Mixograms showed a 64% optimum water absorption for all the treatments (From Figure 5.12 to 5.17). The mixogram prepared with 62% absorption showed a curve with rough edges and wild swings, and the mixogram prepared with 66% absorption appeared slack. After the mixing time pre-test, the optimum absorptions and mixing times were determined and are shown in Table 5.2. Sixty two % absorption, which was lower than that estimated with the mixograph, was used for mixing. The flour with enzyme treatment showed less mixing tolerance, corresponding to the results shown by the farinograph. From the corrected mixing time, it was thought that the increased protein in enzyme treated flour was not functional gluten forming protein.

Table 5.2 Corrected optimum absorption and mixing time by mixing time pre-test

	Treatment	Optimum absorption	Optimum mixing time
1	Control	62%	4 min 15 sec
2	pH5	62%	4 min 15 sec
3	50°C	62%	4 min 15 sec
4	pH5 and 50°C	62%	4 min 15 sec
5	Enzyme, pH5, and 50°C	62%	4 min
6	Enzyme	62%	3 min 30 sec

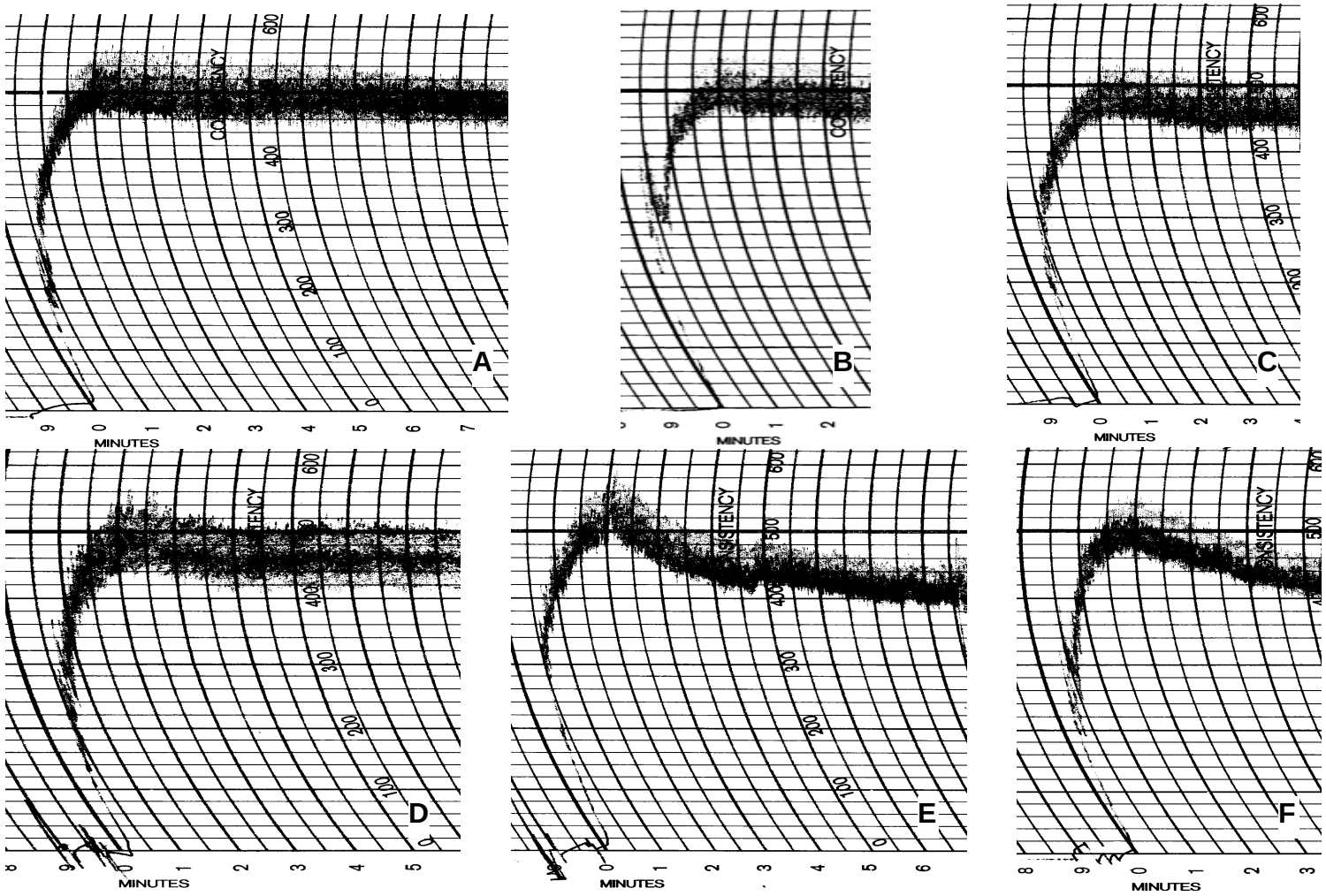


Figure 5.11 Farinograms for the treatments

A) Control (56% absorption); B) pH5 (56%); C) 50°C (56.2%); D) pH5 and 50°C (56%); E) enzyme, pH5, 50°C (56%); F) enzyme (57.2%)

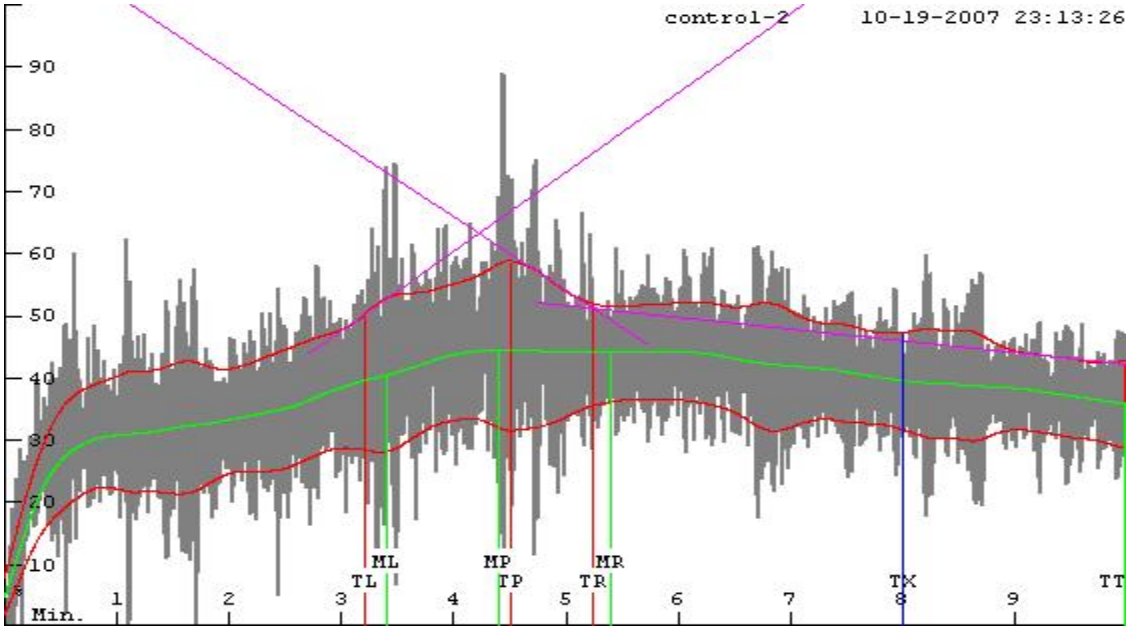


Figure 5.12 Mixogram for the flour from the control wheat (64% absorption)

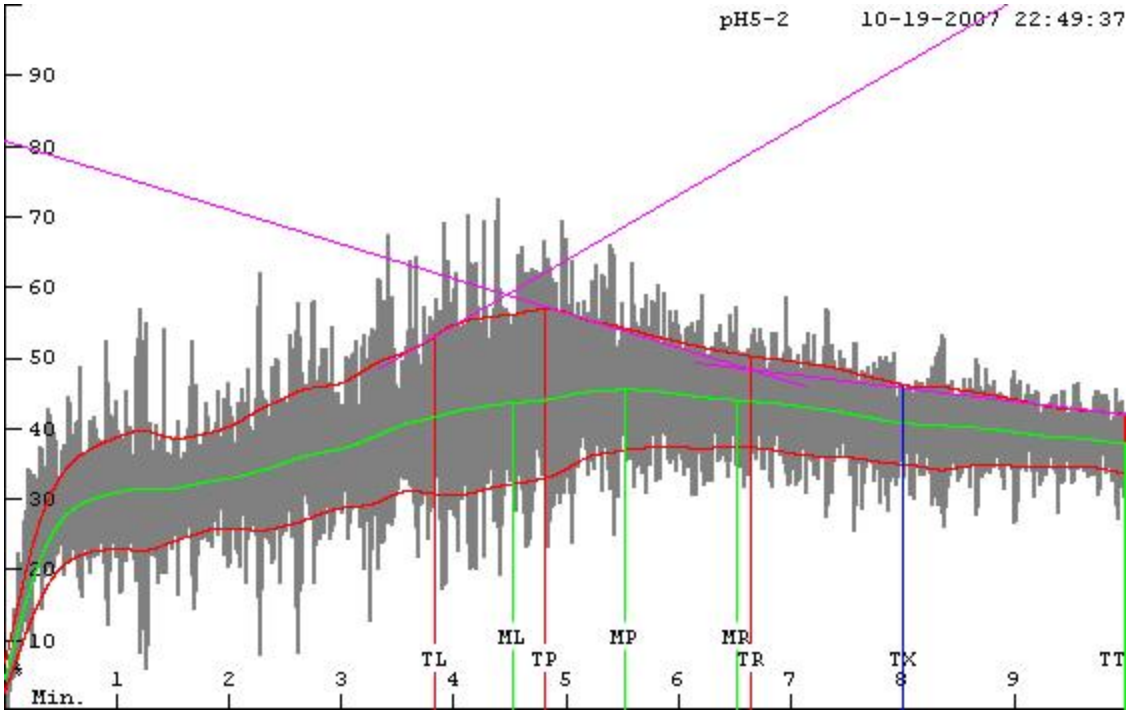


Figure 5.13 Mixogram for the flour from the wheat tempered with pH 5 water (64% absorption)

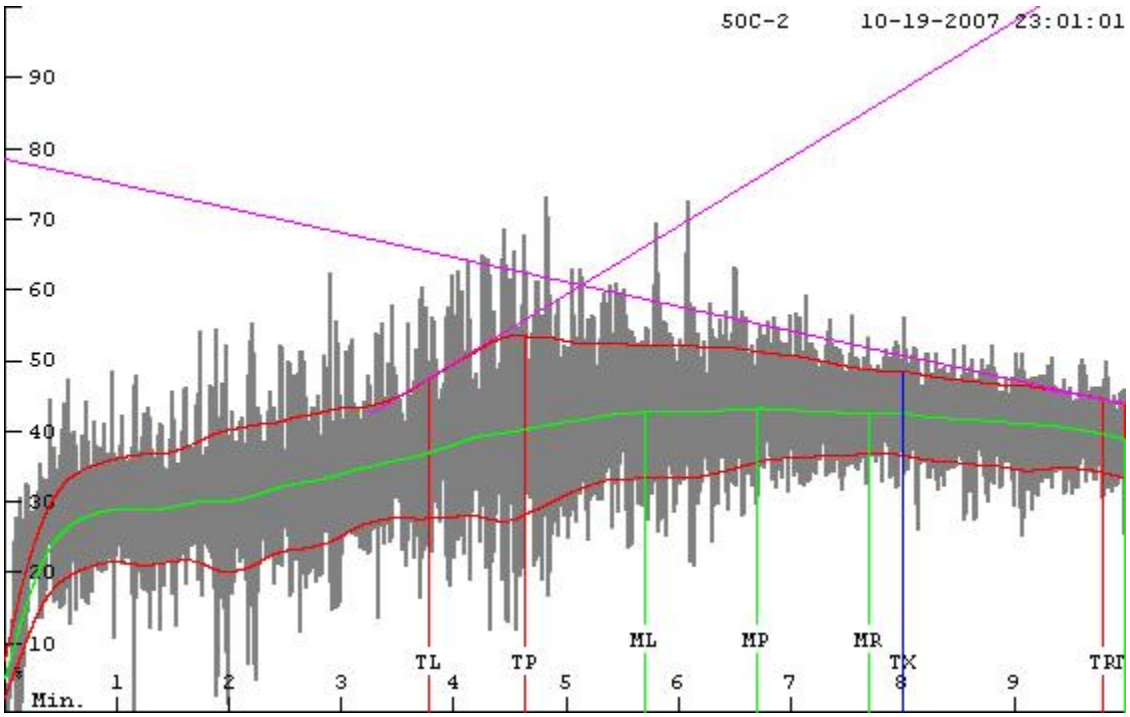


Figure 5.14 Mixogram for the flour from the wheat tempered at 50°C (64% absorption)

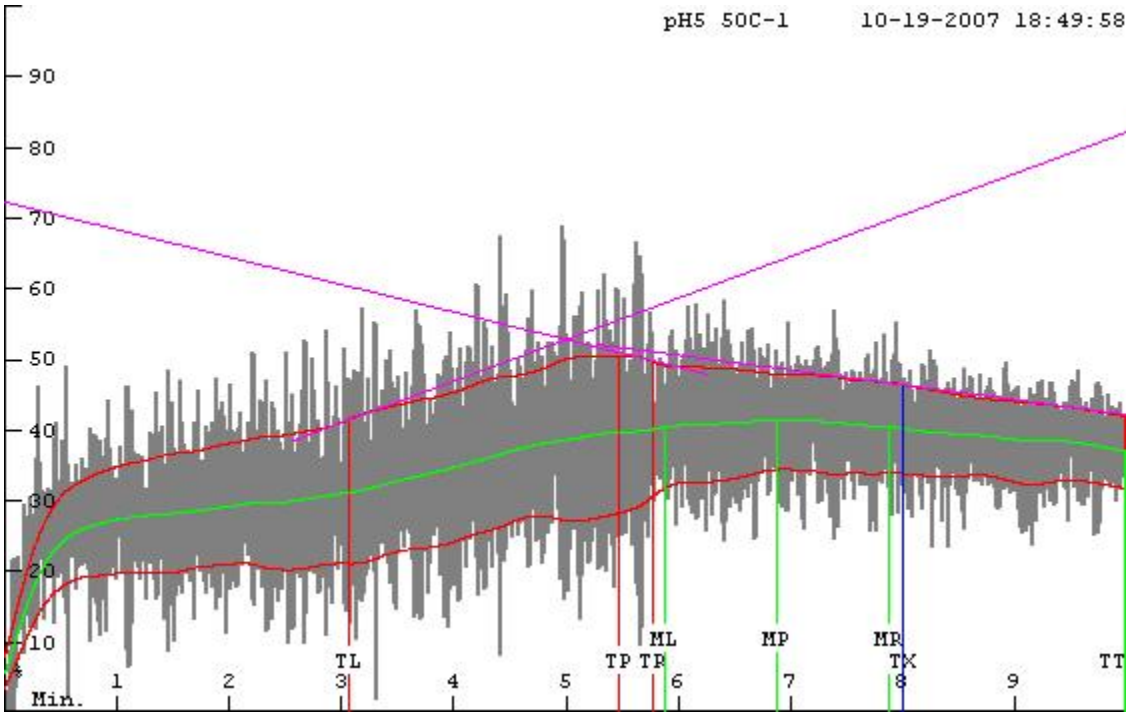


Figure 5.15 Mixogram for the flour from the wheat tempered with pH5 water at 50°C (absorption 64%)

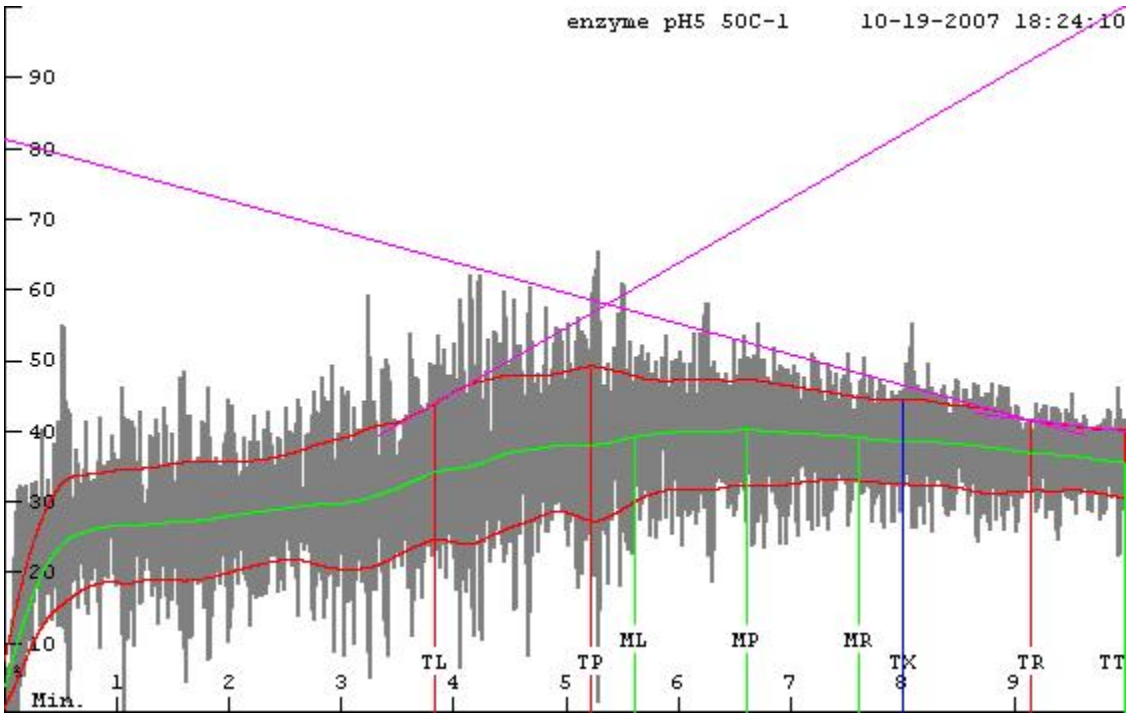


Figure 5.16 Mixogram for the flour from the wheat tempered with pH5 enzyme solution at 50°C (absorption 64%)

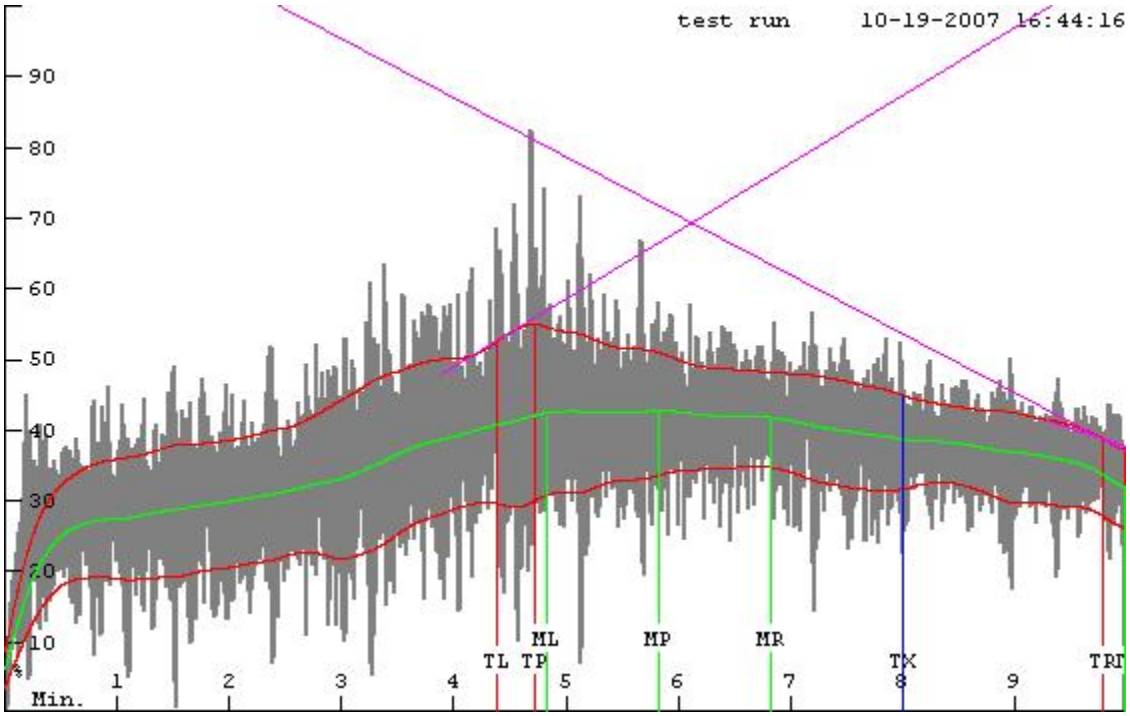


Figure 5.17 Mixogram for the flour from the wheat tempered with enzyme (absorption 64%)

Test baking

The results from the test baking are shown in Table 5.3 and 5.4, and Figure 5.18, and 5.19. There were no significant differences for the treatments in volume or staling rates. There were significant differences in proofing height and bread weight, but the differences were not reflected in the differences in final bread volume. The bread volumes for the treatments were not significantly different for the treatments. The treatment applied during tempering did not make a difference in softness after 24 hours. The bread with flour involving the tempering at 50°C without low pH treatment showed the greatest firmness after 3 and 5 days. Enzyme-only treated bread showed the lowest number in firmness after 1 day. However, enzyme treatment did not seem to slow down the staling rate over time, which was not in the agreement with the previous report (Courtin, C. M., and Delcour, 2002).

Figure 5.18 One lb loaf bread with different treatments

Control; pH5; 50°C; pH5 and 50°C; enzyme, pH5, and 50°C; and enzyme treated bread (from left to right)

Table 5.3 Test baking results

	Treatment	Proofing height (cm)	Bread weight (g)	Bread volume (cc)	Specific volume (cc/g)
1	Control	6.05 [a,b]	143.35 [a,b]	752.50 [a]	5.250 [a]
2	pH5	6.10 [a,b]	142.15 [a,b]	766.65 [a]	5.394 [a]
3	50°C	6.35 [a]	143.65 [a,b]	807.50 [a]	5.620 [a]
4	pH5 and 50°C	6.15 [a]	144.35 [a]	758.30 [a]	5.254 [a]
5	Enzyme, pH5, and 50°C	5.60 [b]	144.50 [a]	735.85 [a]	5.091 [a]
6	Enzyme	6.20 [a]	141.30 [b]	789.15 [a]	5.586 [a]

Means for a given parameter sharing the same letter are not significantly different at $p<0.05$ (n=2).

Table 5.4 Staling test with Voland Stevens Texture Analyser

	Treatment	Load, gram		
		1 day	3 days	5 days
1	Control	337.5 [a]	714.0 [a]	769.0 [b]
2	pH5	335.0 [a]	623.5 [a,b]	903.0 [a]
3	50°C	328.5 [a]	529.5 [b]	740.5 [b]
4	pH5 and 50°C	334.0 [a]	580.5 [a,b]	891.0 [a]
5	Enzyme, pH5, and 50°C	340.5 [a]	601.5 [a,b]	904.5 [a]
6	Enzyme	282.0 [a]	548.0 [b]	852.0 [a]

Means for a given parameter sharing the same letter are not significantly different at $p<0.05$ (n=2).

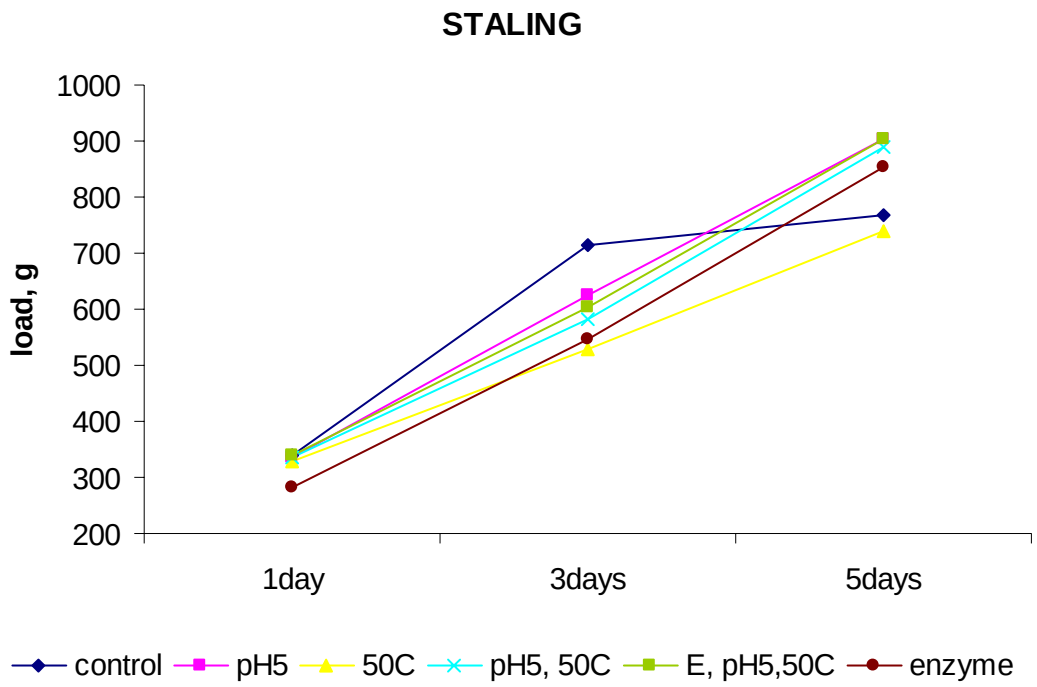


Figure 5.19 Staling rate for breads with different treatments

Conclusions

Cell wall degrading enzymes; cellulase, xylanase, and pectinase were used in this study to hydrolyze the bran fraction and release the sugar. Five independent variables; enzyme concentration, incubation time, incubation temperature, wheat moisture content, and temper water pH were selected to assess their effects on the efficacy of bran removal during wheat milling. When three enzymes were combined and added in temper water under various conditions, no improvements in flour yields were observed. Six equations were arrived at to predict the product yield.

Enzymes seemed to harden the wheat kernel after tempering. Flour milled from the enzymatic tempered wheat contained significantly higher protein compared with flour from the non-enzymatic tempered wheat when the rest of experimental conditions remained the same. It was unclear if the protein came from the wheat kernel or from the enzyme. Although the treatments showed slight differences in mixing time between enzyme treated flour and non-enzyme treated flours, specific loaf volumes. However the firmness of enzyme treated bread was significantly higher than control bread after 5 days storage.

Enzyme penetration within the wheat kernel and its spatial distribution were not understood. Future work could include a study on the degree of enzyme penetration within the cross section of a wheat kernel. Scarifying of the kernel prior to enzyme application may improve enzyme penetration. Also, a study of the change in mechanical and biochemical characteristics of the enzyme treated kernel could give some insight into “if and how” enzymes could ease bran separation. Perhaps a shorter tempering time, allowing the temper water and enzymes to penetrate only into the bran layer, could loosen the bran, making its removal easier than by the existing method.

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Appendix A - Experimental Mill Data Sheet Reported in Grams

EXPERIMENTAL MILL DATA SHEET REPORTED IN GRAMS

DATE: _____

Relative humidity and Temperature: _____

NAME OF MILLER (GRINDER): _____

CO-WORKER: _____

Test #: _____

	1BK	2BK	3BK	4BK	SIZ	1M	1T	2M	3M	2T	4M	5M	LG
OV. 20WW:													
OV. 50GG:													
OV. 70GG:													
OV. 10XX:													
THRU 10XX:													
% HUNG:													
IN:													
OUT:													
LOSS:													

		% Hung
Pat. Flour	**(2BK, 3BK, SIZ, 1M, 1T, 2M, 3M, 4M)**	
1st Clear	**(1BK, 2T, 5M)**	
2nd Clear	**(4BK, LG)**	
St. Grade flour		
Red Dog, Germ, & Shorts		
Bran		
Total		
% Gain or loss (circle one)		
flour yield (based on the total product)		

Bran	
Germ	
Shorts	
Red Dog	

% BREAK RELEASE

1ST: _____
2ND: _____
3RD: _____
4TH: _____

Agtron

Pat. Flour _____
Clear _____

Characteristics of tempered wheat using SKCS

	weight (mg)	diameter (mm)	hardness	MC (%)
1				
2				
average				
std.dv.				

Appendix B - Physical Characteristics of Tempered Wheat (SKCS Data)

Treatment						weight (mg)		diameter (mm)		hardness index		moisture content (%)	
Test #	Enzyme	time	Temp	MC	pH	average	Stdev.	average	Stdev.	average	Stdev.	average	Stdev.
						35.28	1.13	2.82	0.04	62.79	1.24	15.67	0.09
2	60	9	47.5	22	6	34.64	0.88	2.82	0.03	63.69	0.04	15.86	0.06
3	120	12	40.0	20	5	35.47	0.19	2.82	0.01	60.59	0.82	15.48	0.08
4	120	12	25.0	20	5	34.86	0.14	2.77	0.00	59.70	1.66	15.32	0.01
5	180	15	32.5	18	6	35.58	0.09	2.83	0.01	66.23	1.10	15.99	0.01
6	60	15	47.5	18	6	34.55	0.37	2.76	0.00	61.26	0.14	15.42	0.09
7	60	9	32.5	18	6	35.20	0.45	2.79	0.03	59.03	0.28	15.67	0.09
8	120	6	40.0	20	5	35.04	0.35	2.81	0.01	62.12	0.91	15.66	0.07
9	120	12	40.0	16	5	35.13	0.42	2.82	0.02	68.10	1.63	15.95	0.14
10	180	9	32.5	22	6	34.96	0.42	2.79	0.01	62.17	0.55	15.61	0.02
11	120	12	40.0	20	5	35.41	0.08	2.81	0.01	63.53	0.92	15.67	0.09
12	180	15	47.5	18	4	35.86	0.18	2.81	0.01	61.50	1.98	15.86	0.04
13	120	12	40.0	20	5	35.65	0.35	2.82	0.02	59.81	0.92	15.86	0.04
14	60	15	47.5	22	4	35.84	0.06	2.82	0.02	59.95	0.91	16.12	0.12
15	60	15	32.5	18	4	35.55	1.30	2.78	0.04	58.04	1.18	15.80	0.16
16	120	18	40.0	20	5	36.16	1.07	2.85	0.04	61.00	0.21	15.68	0.09
17	0	12	40.0	20	5	35.47	0.15	2.78	0.01	61.32	0.28	15.94	0.08
18	120	12	40.0	20	5	35.53	0.37	2.86	0.01	64.95	2.32	16.42	0.04
19	120	12	40.0	20	5	36.15	0.40	2.88	0.02	63.52	0.88	16.03	0.00
20	120	12	40.0	20	5	35.09	0.49	2.78	0.01	60.79	2.22	15.92	0.11
21	180	15	32.5	22	4	35.53	0.72	2.83	0.04	59.43	1.48	15.98	0.06
22	180	9	32.5	18	4	36.59	0.35	2.86	0.01	59.55	1.00	15.71	0.01
23	180	9	47.5	18	6	36.03	0.85	2.84	0.01	64.46	1.14	15.91	0.04
24	120	12	40.0	20	3	35.55	0.23	2.80	0.01	60.94	1.97	15.52	0.06
25	240	12	40.0	20	5	36.32	0.30	2.91	0.00	63.71	1.52	15.97	0.03
26	180	15	47.5	22	6	36.62	0.35	2.90	0.03	60.32	0.49	16.06	0.01
27	60	9	47.5	18	4	35.31	0.87	2.78	0.07	60.77	0.88	15.40	0.10
28	120	12	40.0	24	5	35.42	0.51	2.82	0.03	61.18	0.05	15.61	0.04
29	60	9	32.5	22	4	35.51	0.26	2.79	0.02	58.73	0.59	16.29	0.08
30	60	15	32.5	22	6	35.22	0.23	2.79	0.01	59.57	0.59	16.09	0.03
31	120	12	40.0	20	5	35.26	0.37	2.80	0.01	62.40	0.88	15.84	0.08
32	120	12	55.0	20	5	35.23	0.13	2.90	0.11	66.95	0.58	16.14	0.04
33	180	9	47.5	22	4	35.04	0.77	2.81	0.03	67.07	0.96	15.97	0.10

Appendix C - Milling Data

Test #	Ash content, %		Protein content, %		Agtron reading		Milling product yield, %						
	patent	clear	patent	clear	patent	clear	patent flour	1st clear	2nd clear	red dog, germ, shorts	bran	feed	yield
1	0.536	0.860	10.785	11.685	72	53	61.0	5.1	1.9	18.0	7.3	25.3	72.9
2	0.408	0.894	10.681	12.297	74	57	60.6	5.6	1.7	16.5	8.3	24.8	73.3
3	0.396	0.640	10.614	10.809	76	64	61.4	6.3	1.9	15.8	11.2	27.0	72.1
4	0.438	0.775	10.676	11.116	76	63	60.3	6.9	2.5	16.7	9.2	25.9	72.9
5	0.403	0.569	10.497	10.983	74	63	56.5	7.4	3.0	20.1	9.6	29.7	69.3
6	0.406	0.755	10.619	11.744	78	63	59.4	4.7	2.3	14.3	11.2	25.5	72.3
7	0.415	0.831	10.555	11.648	79	60	62.5	5.4	2.2	16.6	8.7	25.3	73.5
8	0.451	0.777	10.764	11.053	76	61	61.7	5.8	2.2	14.7	11.1	25.8	73.0
9	0.500	0.831	10.923	11.376	75	60	61.7	6.0	1.8	14.9	10.2	25.1	73.4
10	0.421	0.740	10.749	11.477	76	60	59.6	6.4	2.5	14.5	11.4	25.9	72.6
11	0.420	0.842	10.810	11.160	77	60	62.9	6.1	1.2	15.7	9.3	25.0	73.8
12	0.430	0.755	10.784	11.336	74	58	61.0	7.1	2.9	19.3	7.5	26.8	72.5
13	0.457	0.638	10.664	10.829	77	64	59.5	6.8	2.6	15.9	11.7	27.6	71.4
14	0.520	0.702	10.742	10.205	77	63	63.2	5.8	1.0	13.9	12.1	26.0	72.9
15	0.460	0.694	10.706	11.004	77	66	59.3	7.0	2.2	14.7	11.7	26.4	72.2
16	0.460	0.805	10.789	11.011	78	61	61.9	6.2	1.9	14.5	11.2	25.7	73.1
17	0.429	0.636	10.499	10.808	78	68	60.0	6.1	2.1	14.4	13.2	27.6	72.1
18	0.408	0.661	10.549	10.433	79	63	60.8	5.9	2.5	17.1	11.3	28.4	71.0
19	0.382	0.685	10.374	11.267	77	63	59.2	6.3	3.1	18.5	10.6	29.1	70.2
20	0.416	0.825	10.653	11.513	78	58	60.6	6.2	3.1	17.5	9.5	27.0	72.1
21	0.439	0.740	10.739	10.244	73	63	61.1	6.2	1.6	14.4	13.4	27.8	71.2
22	0.433	0.767	10.761	11.052	75	59	61.8	6.2	1.8	14.6	12.2	26.8	72.2
23	0.466	0.808	10.618	11.436	76	56	61.9	5.7	2.4	16.2	10.9	27.1	72.1
24	0.400	1.010	10.776	11.291	77	56	62.6	6.1	2.2	17.2	8.7	25.9	73.3
25	0.425	0.832	10.591	11.184	73	51	59.1	6.6	2.7	20.1	8.3	28.4	70.7
26	0.432	0.747	10.895	10.426	75	61	61.8	6.5	1.6	14.9	11.6	26.5	72.5
27	0.496	0.876	10.801	11.427	75	59	61.4	6.7	2.2	16.3	9.4	25.7	73.2
28	0.485	0.735	10.878	10.382	75	57	62.9	6.1	1.4	14.7	11.1	25.8	73.2
29	0.423	0.642	10.701	10.067	73	64	62.0	5.9	1.5	14.0	12.9	26.9	72.0
30	0.457	0.862	10.665	11.446	77	59	61.1	5.4	2.5	18.0	8.4	26.4	72.3
31	0.502	0.936	10.810	11.637	75	54	61.9	6.0	2.3	16.9	7.8	24.7	74.0
32	0.503	0.914	10.794	11.635	75	58	60.8	6.3	2.2	17.6	7.9	25.5	73.1
33	0.486	0.935	10.833	11.510	75	53	61.9	6.0	2.1	17.9	7.6	25.5	73.3

Appendix D - SAS data

Minolta Color Analysis for the Clear flour

The RSREG Procedure

Response Surface for Variable MCcolor

Response Mean	10.828485
Root MSE	0.425254
R-Square	0.7145
Coefficient of Variation	3.9272

Regression	DF	Type I Sum of Squares	R-Square	F Value	Pr > F
Linear	5	3.663188	0.4819	4.05	0.0219
Quadratic	5	0.412988	0.0543	0.46	0.8009
Crossproduct	10	1.355562	0.1783	0.75	0.6715
Total Model	20	5.431738	0.7145	1.50	0.2369

Residual	DF	Sum of Squares	Mean Square
Total Error	12	2.170087	0.180841

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	17.750118	13.397538	1.32	0.2099
a	1	-0.015981	0.023679	-0.67	0.5125
b	1	0.086262	0.491032	0.18	0.8635
c	1	0.180868	0.203403	0.89	0.3914
d	1	-1.159589	0.898766	-1.29	0.2213
e	1	0.795931	1.511193	0.53	0.6080
a*a	1	-0.000001889	0.000021487	-0.09	0.9314
b*a	1	-0.000226	0.000591	-0.38	0.7090
b*b	1	0.006467	0.008595	0.75	0.4663
c*a	1	-0.000098611	0.000236	-0.42	0.6838
c*b	1	-0.000750	0.004725	-0.16	0.8765
c*c	1	-0.000654	0.001375	-0.48	0.6428
d*a	1	0.001745	0.000886	1.97	0.0724
d*b	1	-0.008438	0.017719	-0.48	0.6425
d*c	1	0.000041667	0.007088	0.01	0.9954
d*d	1	0.022362	0.019339	1.16	0.2700
e*a	1	-0.001802	0.001772	-1.02	0.3292
e*b	1	-0.017708	0.035438	-0.50	0.6263
e*c	1	-0.018583	0.014175	-1.31	0.2144
e*d	1	0.010937	0.053157	0.21	0.8404
e*e	1	0.030699	0.077354	0.40	0.6984

The RSREG Procedure

Factor	DF	Sum of Squares	Mean Square	F Value	Pr > F
a	6	1.624477	0.270746	1.50	0.2595
b	6	1.367927	0.227988	1.26	0.3439
c	6	0.716811	0.119468	0.66	0.6828
d	6	1.963922	0.327320	1.81	0.1797
e	6	1.116161	0.186027	1.03	0.4528

The RSREG Procedure
Canonical Analysis of Response Surface

Factor	Critical Value
a	218.222345
b	19.454567
c	32.945685
d	19.705237
e	5.514043

Predicted value at stationary point: 10.594366

Eigenvalues	Eigenvectors a	b	c	d	e
0.038218	-0.010666	-0.286617	-0.202439	0.371813	0.859366
0.020410	0.055147	-0.045658	0.164829	0.911458	-0.370067
0.004373	-0.016636	0.911403	-0.343489	0.169487	0.149521
-0.000033603	0.993830	-0.012905	-0.102012	-0.041530	0.001968
-0.004095	0.094180	0.291450	0.896359	-0.023411	0.319656

Stationary point is a saddle point.

The RSREG Procedure

Estimated Ridge of Minimum Response for Variable MCcolor

Radius	Estimated Response	Standard Error
0	10.730490	0.157548
0.100000	10.711206	0.157483
0.200000	10.692370	0.157302
0.300000	10.673971	0.157037
0.400000	10.656001	0.156736
0.500000	10.638450	0.156461
0.600000	10.621307	0.156288
0.700000	10.604562	0.156302
0.800000	10.588204	0.156595
0.900000	10.572223	0.157264
1.000000	10.556608	0.158406
1.100000	10.541348	0.160114
1.200000	10.526431	0.162472
1.300000	10.511846	0.165554
1.400000	10.497581	0.169417
1.500000	10.483624	0.174099
1.600000	10.469963	0.179621
1.700000	10.456585	0.185987
1.800000	10.443478	0.193185
1.900000	10.430630	0.201188
2.000000	10.418028	0.209962

Estimated Ridge of Minimum Response for Variable MCcolor

Radius	Factor Values a	b	c	d	e
0	120.000000	12.000000	40.000000	20.000000	5.000000
0.100000	119.998416	12.037525	39.991075	20.052177	4.923926
0.200000	119.996519	12.075359	39.980242	20.105502	4.849044
0.300000	119.994291	12.113506	39.967413	20.159880	4.775367
0.400000	119.991711	12.151965	39.952497	20.215210	4.702904
0.500000	119.988760	12.190729	39.935402	20.271388	4.631664
0.600000	119.985417	12.229789	39.916034	20.328304	4.561650
0.700000	119.981662	12.269127	39.894301	20.385844	4.492864
0.800000	119.977476	12.308720	39.870109	20.443893	4.425306
0.900000	119.972839	12.348537	39.843368	20.502329	4.358972
1.000000	119.967732	12.388539	39.813989	20.561030	4.293855
1.100000	119.962139	12.428680	39.781885	20.619871	4.229945
1.200000	119.956042	12.468903	39.746977	20.678726	4.167229
1.300000	119.949427	12.509144	39.709189	20.737468	4.105693
1.400000	119.942282	12.549327	39.668454	20.795969	4.045317

1.500000	119.934596	12.589368	39.624713	20.854104	3.986081

The RSREG Procedure

Estimated Ridge of Minimum Response for Variable MCcolor

	Factor Values				
Radius	a	b	c	d	e
1.600000	119.926362	12.629177	39.577919	20.911749	3.927958
1.700000	119.917574	12.668652	39.528037	20.968783	3.870921
1.800000	119.908231	12.707687	39.475043	21.025089	3.814939
1.900000	119.898335	12.746169	39.418930	21.080557	3.759979
2.000000	119.887892	12.783982	39.359705	21.135080	3.706006

```
data a;
input a b c d e MCclolr;
cards;
120   12    40.0  20    7           10.93
60    9     47.5  22    6           11.07
120   12    40.0  20    5           10.46
120   12    25.0  20    5           10.52
180   15    32.5  18    6           10.74
60    15    47.5  18    6           11.27
60    9     32.5  18    6           11.52
120   6     40.0  20    5           11.59
120   12    40.0  16    5           11.84
180   9     32.5  22    6           11.47
120   12    40.0  20    5           11.11
180   15    47.5  18    4           10.78
120   12    40.0  20    5           10.04
60    15    47.5  22    4           10.17
60    15    32.5  18    4           10.42
120   18    40.0  20    5           10.56
0     12    40.0  20    5           10.08
120   12    40.0  20    5           10.43
120   12    40.0  20    5           10.92
120   12    40.0  20    5           10.80
180   15    32.5  22    4           10.35
180   9     32.5  18    4           10.60
180   9     47.5  18    6           11.12
120   12    40.0  20    3           11.00
240   12    40.0  20    5           11.55
180   15    47.5  22    6           10.68
60    9     47.5  18    4           11.16
120   12    40.0  24    5           10.56
60    9     32.5  22    4           9.85
60    15    32.5  22    6           10.47
120   12    40.0  20    5           11.13
120   12    55.0  20    5           10.87
180   9     47.5  22    4           11.28
;
proc rsreg;
model MCcolor=a b c d e/nocode;
ridge min  radius = 0 to 2 by .1;
run;
```

Product Yield of 1st Clear Flour

The RSREG Procedure

Response Surface for Variable yield

Response Mean	6.145455
Root MSE	0.271645
R-Square	0.9134
Coefficient of Variation	4.4203

Regression	DF	Type I Sum of Squares	R-Square	F Value	Pr > F
Linear	5	3.853333	0.3770	10.44	0.0005
Quadratic	5	1.202995	0.1177	3.26	0.0434
Crossproduct	10	4.280000	0.4187	5.80	0.0028
Total Model	20	9.336328	0.9134	6.33	0.0011

Residual	DF	Sum of Squares	Mean Square
Total Error	12	0.885490	0.073791

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	12.212908	8.558119	1.43	0.1791
a	1	-0.036152	0.015125	-2.39	0.0341
b	1	0.751144	0.313663	2.39	0.0338
c	1	-0.140131	0.129930	-1.08	0.3020
d	1	-0.262377	0.574116	-0.46	0.6558
e	1	-0.801961	0.965324	-0.83	0.4223
a*a	1	0.000010008	0.000013726	0.73	0.4799
b*a	1	0.001250	0.000377	3.31	0.0062
b*b	1	-0.005719	0.005490	-1.04	0.3181
c*a	1	0	0.000151	0.00	1.0000
c*b	1	-0.005556	0.003018	-1.84	0.0905
c*c	1	0.001752	0.000878	1.99	0.0694
d*a	1	-0.000104	0.000566	-0.18	0.8570
d*b	1	-0.022917	0.011319	-2.02	0.0657
d*c	1	0.007500	0.004527	1.66	0.1235
d*d	1	-0.009743	0.012353	-0.79	0.4456
e*a	1	0.005000	0.001132	4.42	0.0008
e*b	1	-0.008333	0.022637	-0.37	0.7192
e*c	1	-0.020000	0.009055	-2.21	0.0474
e*d	1	0.118750	0.033956	3.50	0.0044
e*e	1	-0.151471	0.049413	-3.07	0.0098

The RSREG Procedure

Factor	DF	Sum of Squares	Mean Square	F Value	Pr > F
a	6	3.791732	0.631955	8.56	0.0009
b	6	1.827565	0.304594	4.13	0.0176
c	6	1.480899	0.246816	3.34	0.0356
d	6	1.657565	0.276261	3.74	0.0247
e	6	4.807565	0.801261	10.86	0.0003

The RSREG Procedure
Canonical Analysis of Response Surface

Factor	Critical Value
a	82.329561
b	7.590234

c	33.235118
d	23.425707
e	5.491282

Predicted value at stationary point: 5.971684

	Eigenvectors				
Eigenvalues	a	b	c	d	e
0.018216	0.022719	-0.459549	0.086243	0.832018	0.297668
0.002722	-0.123485	-0.112051	0.972577	-0.120526	-0.108461
0.000054435	0.989473	0.059368	0.131666	-0.008791	0.002559
-0.012423	-0.070672	0.879053	0.160092	0.420605	0.140478
-0.173739	-0.013603	0.001067	0.060753	-0.340930	0.938024

Stationary point is a saddle point.

The RSREG Procedure

Estimated Ridge of Maximum Response for Variable yield

Radius	Estimated Response	Standard Error
0	6.223529	0.100639
0.100000	6.247398	0.100582
0.200000	6.269270	0.100432
0.300000	6.289595	0.100234
0.400000	6.308820	0.100038
0.500000	6.327325	0.099889
0.600000	6.345402	0.099821
0.700000	6.363262	0.099867
0.800000	6.381054	0.100056
0.900000	6.398883	0.100417
1.000000	6.416823	0.100981
1.100000	6.434928	0.101777
1.200000	6.453239	0.102837
1.300000	6.471784	0.104190
1.400000	6.490587	0.105865
1.500000	6.509666	0.107888
1.600000	6.529035	0.110283
1.700000	6.548704	0.113070
1.800000	6.568683	0.116265
1.900000	6.588978	0.119881
2.000000	6.609597	0.123928

Estimated Ridge of Maximum Response for Variable yield

	Factor Values				
Radius	a	b	c	d	e
0	120.000000	12.000000	40.000000	20.000000	5.000000
0.100000	120.001631	12.018797	39.993382	19.974965	4.905272
0.200000	120.003145	12.042118	39.986923	19.935764	4.815829
0.300000	120.004467	12.069859	39.980708	19.882862	4.733530
0.400000	120.005547	12.101339	39.974774	19.818626	4.659168
0.500000	120.006369	12.135644	39.969098	19.746189	4.592342
0.600000	120.006944	12.171954	39.963623	19.668332	4.531965
0.700000	120.007296	12.209669	39.958285	19.587068	4.476806
0.800000	120.007453	12.248392	39.953028	19.503713	4.425771
0.900000	120.007437	12.287862	39.947809	19.419094	4.377983
1.000000	120.007271	12.327910	39.942593	19.333722	4.332766
1.100000	120.006972	12.368423	39.937358	19.247913	4.289607
1.200000	120.006555	12.409322	39.932086	19.161867	4.248113
1.300000	120.006031	12.450550	39.926766	19.075708	4.207984
1.400000	120.005412	12.492065	39.921389	18.989515	4.168987
1.500000	120.004706	12.533835	39.915949	18.903339	4.130938

The RSREG Procedure

Estimated Ridge of Maximum Response for Variable yield

Factor Values

Radius	a	b	c	d	e
1.600000	120.003920	12.575832	39.910444	18.817211	4.093693
1.700000	120.003063	12.618035	39.904870	18.731152	4.057133
1.800000	120.002138	12.660426	39.899227	18.645172	4.021164
1.900000	120.001152	12.702989	39.893515	18.559278	3.985709
2.000000	120.000109	12.745712	39.887734	18.473474	3.950702

```
data a;
input a b c d e yield;
cards;
120   12    40.0  20    7           5.1
60    9     47.5  22    6           5.6
120   12    40.0  20    5           6.3
120   12    25.0  20    5           6.9
180   15    32.5  18    6           7.4
60    15    47.5  18    6           4.7
60    9     32.5  18    6           5.4
120   6     40.0  20    5           5.8
120   12    40.0  16    5           6.0
180   9     32.5  22    6           6.4
120   12    40.0  20    5           6.1
180   15    47.5  18    4           7.1
120   12    40.0  20    5           6.8
60    15    47.5  22    4           5.8
60    15    32.5  18    4           7.0
120   18    40.0  20    5           6.2
0     12    40.0  20    5           6.1
120   12    40.0  20    5           5.9
120   12    40.0  20    5           6.3
120   12    40.0  20    5           6.2
180   15    32.5  22    4           6.2
180   9     32.5  18    4           6.2
180   9     47.5  18    6           5.7
120   12    40.0  20    3           6.1
240   12    40.0  20    5           6.6
180   15    47.5  22    6           6.5
60    9     47.5  18    4           6.7
120   12    40.0  24    5           6.1
60    9     32.5  22    4           5.9
60    15    32.5  22    6           5.4
120   12    40.0  20    5           6.0
120   12    55.0  20    5           6.3
180   9     47.5  22    4           6.0
;
proc rsreg;
model yield=a b c d e/nocode;
ridge max  radius = 0 to 2 by .1;
run;
```

Product Yield of Shorts, Red Dog, and Germ

The RSREG Procedure

Response Surface for Variable RGSyield

Response Mean	16.254545
Root MSE	1.039779
R-Square	0.8706
Coefficient of Variation	6.3968

Regression	DF	Type I Sum of Squares	R-Square	F Value	Pr > F
Linear	5	21.405000	0.2135	3.96	0.0236
Quadratic	5	19.628142	0.1958	3.63	0.0312
Crossproduct	10	46.235000	0.4612	4.28	0.0102
Total Model	20	87.268142	0.8706	4.04	0.0083

Residual	DF	Sum of Squares	Mean Square
Total Error	12	12.973676	1.081140

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-88.871977	32.758046	-2.71	0.0189
a	1	0.064906	0.057896	1.12	0.2842
b	1	3.468464	1.200613	2.89	0.0136
c	1	0.733366	0.497336	1.47	0.1661
d	1	5.785907	2.197554	2.63	0.0219
e	1	3.167157	3.694986	0.86	0.4082
a*a	1	0.000026144	0.000052538	0.50	0.6277
b*a	1	0.002778	0.001444	1.92	0.0785
b*b	1	-0.063154	0.021015	-3.01	0.0110
c*a	1	0.000972	0.000578	1.68	0.1182
c*b	1	-0.033333	0.011553	-2.89	0.0137
c*c	1	0.001229	0.003362	0.37	0.7211
d*a	1	-0.004688	0.002166	-2.16	0.0514
d*b	1	-0.066667	0.043324	-1.54	0.1498
d*c	1	0.009167	0.017330	0.53	0.6065
d*d	1	-0.129596	0.047284	-2.74	0.0179
e*a	1	-0.007292	0.004332	-1.68	0.1182
e*b	1	0.083333	0.086648	0.96	0.3552
e*c	1	-0.141667	0.034659	-4.09	0.0015
e*d	1	0.043750	0.129972	0.34	0.7422
e*e	1	0.181618	0.189138	0.96	0.3559

The RSREG Procedure

Factor	DF	Sum of Squares	Mean Square	F Value	Pr > F
a	6	30.496879	5.082813	4.70	0.0110
b	6	26.605212	4.434202	4.10	0.0180
c	6	31.306879	5.217813	4.83	0.0100
d	6	19.108824	3.184804	2.95	0.0526
e	6	25.651046	4.275174	3.95	0.0205

The RSREG Procedure
Canonical Analysis of Response Surface

Factor	Critical Value
a	70.991713
b	9.645719

c	45.096513
d	21.088277
e	5.541151

Predicted value at stationary point: 16.478209

	Eigenvectors				
Eigenvalues	a	b	c	d	e
0.213974	-0.016067	0.154727	-0.321942	0.040209	0.933026
0.000174	0.996861	0.048381	-0.052404	-0.033546	-0.007493
-0.021208	0.062951	-0.148585	0.915885	0.150726	0.335256
-0.055075	-0.044592	0.886280	0.230690	-0.395843	-0.051084
-0.147741	0.007681	0.407614	-0.039303	0.904349	-0.119998

Stationary point is a saddle point.

The RSREG Procedure

Estimated Ridge of Minimum Response for Variable RGSyield

Radius	Estimated Response	Standard Error
0	16.794118	0.385216
0.100000	16.758412	0.385037
0.200000	16.723591	0.384582
0.300000	16.688732	0.383993
0.400000	16.653038	0.383385
0.500000	16.615911	0.382835
0.600000	16.576926	0.382402
0.700000	16.535795	0.382138
0.800000	16.492318	0.382098
0.900000	16.446353	0.382343
1.000000	16.397800	0.382938
1.100000	16.346586	0.383957
1.200000	16.292656	0.385476
1.300000	16.235967	0.387576
1.400000	16.176487	0.390340
1.500000	16.114190	0.393852
1.600000	16.049056	0.398197
1.700000	15.981068	0.403456
1.800000	15.910213	0.409707
1.900000	15.836480	0.417021
2.000000	15.759858	0.425464

Estimated Ridge of Minimum Response for Variable RGSyield

	Factor Values				
Radius	a	b	c	d	e
0	120.000000	12.000000	40.000000	20.000000	5.000000
0.100000	119.996152	11.992345	39.989813	20.054802	4.917419
0.200000	119.992105	11.990127	39.973097	20.122897	4.845040
0.300000	119.988259	11.994087	39.952324	20.202063	4.783841
0.400000	119.984881	12.003998	39.930069	20.288954	4.732847
0.500000	119.982061	12.019049	39.908096	20.380584	4.690121
0.600000	119.979775	12.038294	39.887294	20.474874	4.653710
0.700000	119.977953	12.060888	39.867982	20.570543	4.622033
0.800000	119.976516	12.086150	39.850185	20.666840	4.593913
0.900000	119.975397	12.113551	39.833805	20.763339	4.568498
1.000000	119.974537	12.142683	39.818694	20.859799	4.545173
1.100000	119.973889	12.173233	39.804705	20.956089	4.523490
1.200000	119.973417	12.204955	39.791695	21.052142	4.503116
1.300000	119.973091	12.237658	39.779540	21.147925	4.483802
1.400000	119.972888	12.271189	39.768133	21.243429	4.465357
1.500000	119.972788	12.305425	39.757380	21.338657	4.447635

The RSREG Procedure

Estimated Ridge of Minimum Response for Variable RGSyield

Factor Values

Radius	a	b	c	d	e
1.600000	119.972776	12.340267	39.747202	21.433618	4.430518
1.700000	119.972840	12.375632	39.737530	21.528325	4.413914
1.800000	119.972969	12.411454	39.728308	21.622791	4.397749
1.900000	119.973155	12.447676	39.719484	21.717034	4.381962
2.000000	119.973390	12.484251	39.711017	21.811067	4.366504

```
data a;
input a b c d e RGSyield;
cards;
120   12    40.0  20    7           18
60    9     47.5  22    6           16.5
120   12    40.0  20    5           15.8
120   12    25.0  20    5           16.7
180   15    32.5  18    6           20.1
60    15    47.5  18    6           14.3
60    9     32.5  18    6           16.6
120   6     40.0  20    5           14.7
120   12    40.0  16    5           14.9
180   9     32.5  22    6           14.5
120   12    40.0  20    5           15.7
180   15    47.5  18    4           19.3
120   12    40.0  20    5           15.9
60    15    47.5  22    4           13.9
60    15    32.5  18    4           14.7
120   18    40.0  20    5           14.5
0     12    40.0  20    5           14.4
120   12    40.0  20    5           17.1
120   12    40.0  20    5           18.5
120   12    40.0  20    5           17.5
180   15    32.5  22    4           14.4
180   9     32.5  18    4           14.6
180   9     47.5  18    6           16.2
120   12    40.0  20    3           17.2
240   12    40.0  20    5           20.1
180   15    47.5  22    6           14.9
60    9     47.5  18    4           16.3
120   12    40.0  24    5           14.7
60    9     32.5  22    4           14.0
60    15    32.5  22    6           18.0
120   12    40.0  20    5           16.9
120   12    55.0  20    5           17.6
180   9     47.5  22    4           17.9
;
proc rsreg;
model RGSyield=a b c d e/nocode;
ridge min  radius = 0 to 2 by .1;
run;
```

Hardness of Tempered Wheat

The RSREG Procedure

Response Surface for Variable hard

Response Mean	61.974848
Root MSE	2.131031
R-Square	0.7419
Coefficient of Variation	3.4385

Regression	DF	Type I Sum of Squares	R-Square	F Value	Pr > F
Linear	5	87.569854	0.4147	3.86	0.0257
Quadratic	5	14.612785	0.0692	0.64	0.6716
Crossproduct	10	54.464063	0.2579	1.20	0.3773
Total Model	20	156.646701	0.7419	1.72	0.1668

Residual	DF	Sum of Squares	Mean Square
Total Error	12	54.495523	4.541294

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-23.376597	67.137764	-0.35	0.7337
a	1	0.124934	0.118658	1.05	0.3131
b	1	5.687014	2.460661	2.31	0.0394
c	1	0.753583	1.019292	0.74	0.4739
d	1	-0.616979	4.503898	-0.14	0.8933
e	1	13.240833	7.572891	1.75	0.1059
a*a	1	-0.000056424	0.000108	-0.52	0.6098
b*a	1	-0.000823	0.002960	-0.28	0.7857
b*b	1	-0.049097	0.043071	-1.14	0.2766
c*a	1	-0.000601	0.001184	-0.51	0.6207
c*b	1	-0.046528	0.023678	-1.97	0.0730
c*c	1	-0.000011111	0.006891	-0.00	0.9987
d*a	1	-0.002911	0.004440	-0.66	0.5243
d*b	1	-0.162604	0.088793	-1.83	0.0920
d*c	1	0.024958	0.035517	0.70	0.4956
d*d	1	0.082031	0.096910	0.85	0.4139
e*a	1	-0.000448	0.008879	-0.05	0.9606
e*b	1	0.108958	0.177586	0.61	0.5510
e*c	1	-0.090083	0.071034	-1.27	0.2288
e*d	1	-0.329687	0.266379	-1.24	0.2395
e*e	1	-0.365625	0.387638	-0.94	0.3642

The RSREG Procedure

Factor	DF	Sum of Squares	Mean Square	F Value	Pr > F
a	6	29.683591	4.947265	1.09	0.4216
b	6	46.150774	7.691796	1.69	0.2058
c	6	67.702691	11.283782	2.48	0.0847
d	6	37.512935	6.252156	1.38	0.2995
e	6	29.890019	4.981670	1.10	0.4178

The RSREG Procedure
Canonical Analysis of Response Surface

Factor	Critical Value
a	769.096465
b	-14.239580

c	277.358286
d	-40.074826
e	-0.585803

Predicted value at stationary point: 97.166914

	Eigenvectors				
Eigenvalues	a	b	c	d	e
0.182169	-0.005818	-0.389392	0.184214	0.847887	-0.309019
-0.000037060	0.989784	-0.021407	0.136565	-0.034548	-0.005044
-0.000762	-0.142055	-0.065858	0.956579	-0.245255	-0.017029
-0.089090	0.010558	0.915932	0.156849	0.364005	-0.062096
-0.425038	0.001508	-0.068169	0.088152	0.295371	0.948860

Stationary point is a saddle point.

The RSREG Procedure

Estimated Ridge of Maximum Response for Variable hard

Radius	Estimated Response	Standard Error
0	62.471667	0.789503
0.100000	62.543814	0.789134
0.200000	62.612978	0.788157
0.300000	62.680311	0.786820
0.400000	62.746894	0.785391
0.500000	62.813664	0.784131
0.600000	62.881379	0.783289
0.700000	62.950629	0.783112
0.800000	63.021865	0.783854
0.900000	63.095429	0.785774
1.000000	63.171581	0.789142
1.100000	63.250517	0.794233
1.200000	63.332392	0.801323
1.300000	63.417325	0.810683
1.400000	63.505409	0.822572
1.500000	63.596721	0.837233
1.600000	63.691321	0.854882
1.700000	63.789259	0.875708
1.800000	63.890574	0.899866
1.900000	63.995302	0.927476
2.000000	64.103470	0.958626

Estimated Ridge of Maximum Response for Variable hard

	Factor Values				
Radius	a	b	c	d	e
0	120.000000	12.000000	40.000000	20.000000	5.000000
0.100000	120.002425	11.979972	40.023161	19.954919	5.083811
0.200000	120.005061	11.963920	40.045702	19.897291	5.161353
0.300000	120.007810	11.953377	40.066438	19.828438	5.232202
0.400000	120.010566	11.949174	40.084379	19.750612	5.296632
0.500000	120.013246	11.951345	40.098952	19.666297	5.355398
0.600000	120.015794	11.959358	40.110005	19.577676	5.409439
0.700000	120.018186	11.972411	40.117689	19.486415	5.459660
0.800000	120.020416	11.989662	40.122315	19.393671	5.506833
0.900000	120.022491	12.010346	40.124250	19.300206	5.551578
1.000000	120.024424	12.033816	40.123854	19.206505	5.594378
1.100000	120.026228	12.059548	40.121458	19.112860	5.635608
1.200000	120.027918	12.087122	40.117346	19.019447	5.675557
1.300000	120.029508	12.116204	40.111764	18.926362	5.714449
1.400000	120.031008	12.146532	40.104916	18.833654	5.752461
1.500000	120.032431	12.177895	40.096975	18.741342	5.789733

The RSREG Procedure

Estimated Ridge of Maximum Response for Variable hard

Factor Values

Radius	a	b	c	d	e
1.600000	120.033784	12.210125	40.088085	18.649426	5.826376
1.700000	120.035075	12.243087	40.078368	18.557896	5.862481
1.800000	120.036313	12.276672	40.067926	18.466736	5.898122
1.900000	120.037501	12.310789	40.056845	18.375927	5.933359
2.000000	120.038646	12.345366	40.045199	18.285447	5.968243

```
data d;
input a b c d e hard;
cards;
120   12    40.0  20    7           62.79
60    9     47.5  22    6           63.69
120   12    40.0  20    5           60.59
120   12    25.0  20    5           59.70
180   15    32.5  18    6           66.23
60    15    47.5  18    6           61.26
60    9     32.5  18    6           59.03
120   6     40.0  20    5           62.12
120   12    40.0  16    5           68.10
180   9     32.5  22    6           62.17
120   12    40.0  20    5           63.53
180   15    47.5  18    4           61.50
120   12    40.0  20    5           59.81
60    15    47.5  22    4           59.95
60    15    32.5  18    4           58.04
120   18    40.0  20    5           61.00
0     12    40.0  20    5           61.32
120   12    40.0  20    5           64.95
120   12    40.0  20    5           63.52
120   12    40.0  20    5           60.79
180   15    32.5  22    4           59.43
180   9     32.5  18    4           59.55
180   9     47.5  18    6           64.46
120   12    40.0  20    3           60.94
240   12    40.0  20    5           63.71
180   15    47.5  22    6           60.32
60    9     47.5  18    4           60.77
120   12    40.0  24    5           61.18
60    9     32.5  22    4           58.73
60    15    32.5  22    6           59.57
120   12    40.0  20    5           62.40
120   12    55.0  20    5           66.95
180   9     47.5  22    4           67.07
;
proc rsreg;
model hard=a b c d e/nocode;
ridge max  radius = 0 to 2 by .1;
run;
```

Stepwise Regression Analysis for Red Dog, Germ, and Shorts – Step 1

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The RSREG Procedure

Response Surface for Variable RGSyield

Response Mean	16.254545
Root MSE	1.039779
R-Square	0.8706
Coefficient of Variation	6.3968
Sum of Squared Residuals	12.973676471
Predicted Residual SS (PRESS)	181.83982772

Regression	DF	Type I Sum of Squares	R-Square	F Value	Pr > F
Linear	5	21.405000	0.2135	3.96	0.0236
Quadratic	5	19.628142	0.1958	3.63	0.0312
Crossproduct	10	46.235000	0.4612	4.28	0.0102
Total Model	20	87.268142	0.8706	4.04	0.0083

Residual	DF	Sum of Squares	Mean Square	F Value	Pr > F
Lack of Fit	6	6.479391	1.079898	1.00	0.5011
Pure Error	6	6.494286	1.082381		
Total Error	12	12.973676	1.081140		

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The RSREG Procedure

Parameter	DF	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-88.871977	32.758046	-2.71	0.0189
a	1	0.064906	0.057896	1.12	0.2842
b	1	3.468464	1.200613	2.89	0.0136
c	1	0.733366	0.497336	1.47	0.1661
d	1	5.785907	2.197554	2.63	0.0219
e	1	3.167157	3.694986	0.86	0.4082
a*a	1	0.000026144	0.000052538	0.50	0.6277
b*a	1	0.002778	0.001444	1.92	0.0785
b*b	1	-0.063154	0.021015	-3.01	0.0110
c*a	1	0.000972	0.000578	1.68	0.1182
c*b	1	-0.033333	0.011553	-2.89	0.0137
c*c	1	0.001229	0.003362	0.37	0.7211
d*a	1	-0.004688	0.002166	-2.16	0.0514
d*b	1	-0.066667	0.043324	-1.54	0.1498
d*c	1	0.009167	0.017330	0.53	0.6065
d*d	1	-0.129596	0.047284	-2.74	0.0179
e*a	1	-0.007292	0.004332	-1.68	0.1182
e*b	1	0.083333	0.086648	0.96	0.3552
e*c	1	-0.141667	0.034659	-4.09	0.0015
e*d	1	0.043750	0.129972	0.34	0.7422
e*e	1	0.181618	0.189138	0.96	0.3559

Factor	DF	Sum of Squares	Mean Square	F Value	Pr > F
a	6	30.496879	5.082813	4.70	0.0110
b	6	26.605212	4.434202	4.10	0.0180
c	6	31.306879	5.217813	4.83	0.0100
d	6	19.108824	3.184804	2.95	0.0526
e	6	25.651046	4.275174	3.95	0.0205

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The RSREG Procedure
Canonical Analysis of Response Surface

Factor	Critical Value
a	70.991713
b	9.645719
c	45.096513
d	21.088277
e	5.541151

Predicted value at stationary point: 16.478209

Eigenvalues	Eigenvectors a	b	c	d	e
0.213974	-0.016067	0.154727	-0.321942	0.040209	0.933026
0.000174	0.996861	0.048381	-0.052404	-0.033546	-0.007493
-0.021208	0.062951	-0.148585	0.915885	0.150726	0.335256
-0.055075	-0.044592	0.886280	0.230690	-0.395843	-0.051084
-0.147741	0.007681	0.407614	-0.039303	0.904349	-0.119998

Stationary point is a saddle point.

The RSREG Procedure

Estimated Ridge of Minimum Response for Variable RGSyield

Radius	Estimated Response	Standard Error
0	16.794118	0.385216
0.100000	16.758412	0.385037
0.200000	16.723591	0.384582
0.300000	16.688732	0.383993
0.400000	16.653038	0.383385
0.500000	16.615911	0.382835
0.600000	16.576926	0.382402
0.700000	16.535795	0.382138
0.800000	16.492318	0.382098
0.900000	16.446353	0.382343
1.000000	16.397800	0.382938
1.100000	16.346586	0.383957
1.200000	16.292656	0.385476
1.300000	16.235967	0.387576
1.400000	16.176487	0.390340
1.500000	16.114190	0.393852
1.600000	16.049056	0.398197
1.700000	15.981068	0.403456
1.800000	15.910213	0.409707
1.900000	15.836480	0.417021
2.000000	15.759858	0.425464

Estimated Ridge of Minimum Response for Variable RGSyield

Radius	Factor Values a	b	c	d	e
0	120.000000	12.000000	40.000000	20.000000	5.000000
0.100000	119.996152	11.992345	39.989813	20.054802	4.917419
0.200000	119.992105	11.990127	39.973097	20.122897	4.845040
0.300000	119.988259	11.994087	39.952324	20.202063	4.783841
0.400000	119.984881	12.003998	39.930069	20.288954	4.732847
0.500000	119.982061	12.019049	39.908096	20.380584	4.690121
0.600000	119.979775	12.038294	39.887294	20.474874	4.653710
0.700000	119.977953	12.060888	39.867982	20.570543	4.622033
0.800000	119.976516	12.086150	39.850185	20.666840	4.593913
0.900000	119.975397	12.113551	39.833805	20.763339	4.568498
1.000000	119.974537	12.142683	39.818694	20.859799	4.545173
1.100000	119.973889	12.173233	39.804705	20.956089	4.523490
1.200000	119.973417	12.204955	39.791695	21.052142	4.503116
1.300000	119.973091	12.237658	39.779540	21.147925	4.483802
1.400000	119.972888	12.271189	39.768133	21.243429	4.465357
1.500000	119.972788	12.305425	39.757380	21.338657	4.447635

The RSREG Procedure

Estimated Ridge of Minimum Response for Variable RGSyield

Radius	Factor Values a	b	c	d	e
1.600000	119.972776	12.340267	39.747202	21.433618	4.430518
1.700000	119.972840	12.375632	39.737530	21.528325	4.413914
1.800000	119.972969	12.411454	39.728308	21.622791	4.397749
1.900000	119.973155	12.447676	39.719484	21.717034	4.381962
2.000000	119.973390	12.484251	39.711017	21.811067	4.366504

Regression

The REG Procedure
Model: MODEL1
Dependent Variable: RGSyield

Number of Observations Read	33
Number of Observations Used	33

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	87.26814	4.36341	4.04	0.0083
Error	12	12.97368	1.08114		
Corrected Total	32	100.24182			

Root MSE	1.03978	R-Square	0.8706
Dependent Mean	16.25455	Adj R-Sq	0.6549
Coeff Var	6.39685		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-88.87198	32.75805	-2.71	0.0189
a	1	0.06491	0.05790	1.12	0.2842
b	1	3.46846	1.20061	2.89	0.0136
c	1	0.73337	0.49734	1.47	0.1661
d	1	5.78591	2.19755	2.63	0.0219
e	1	3.16716	3.69499	0.86	0.4082
a2	1	0.00002614	0.00005254	0.50	0.6277
b2	1	-0.06315	0.02102	-3.01	0.0110
c2	1	0.00123	0.00336	0.37	0.7211
d2	1	-0.12960	0.04728	-2.74	0.0179
e2	1	0.18162	0.18914	0.96	0.3559
ab	1	0.00278	0.00144	1.92	0.0785
ac	1	0.00097222	0.00057765	1.68	0.1182
ad	1	-0.00469	0.00217	-2.16	0.0514
ae	1	-0.00729	0.00433	-1.68	0.1182
bc	1	-0.03333	0.01155	-2.89	0.0137
bd	1	-0.06667	0.04332	-1.54	0.1498
be	1	0.08333	0.08665	0.96	0.3552
cd	1	0.00917	0.01733	0.53	0.6065
ce	1	-0.14167	0.03466	-4.09	0.0015
de	1	0.04375	0.12997	0.34	0.7422

Regression/selection=stepwise

The REG Procedure
Model: MODEL1
Dependent Variable: RGSyield

Number of Observations Read	33
Number of Observations Used	33

Stepwise Selection: Step 1

Variable ac Entered: R-Square = 0.1711 and C(p) = 47.8579

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	17.14766	17.14766	6.40	0.0167
Error	31	83.09416	2.68046		
Corrected Total	32	100.24182			

Variable	Parameter Estimate	Standard Error	Type II SS	F Value	Pr > F
Intercept	14.68766	0.68191	1243.52954	463.92	<.0001
ac	0.00032644	0.00012906	17.14766	6.40	0.0167

Bounds on condition number: 1, 1

All variables left in the model are significant at the 0.1500 level.

No other variable met the 0.1500 significance level for entry into the model.

Summary of Stepwise Selection

Step	Variable Entered	Variable Removed	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
1	ac		1	0.1711	0.1711	47.8579	6.40	0.0167

Regression/selection=R^2

The REG Procedure
Model: MODEL1
Dependent Variable: RGSyield

R-Square Selection Method

Number of Observations Read 33
Number of Observations Used 33

Number in Model	R-Square	Variables in Model
1	0.1711	ac
1	0.1600	ab
1	0.1544	a2
1	0.1501	a
1	0.1492	ae
1	0.1071	ad
1	0.0349	d2
1	0.0293	d
1	0.0282	e2
1	0.0240	e
1	0.0224	be
1	0.0152	ce
1	0.0084	c2
1	0.0073	c
1	0.0041	de
1	0.0032	bc
1	0.0028	b
1	0.0007	bd
1	0.0001	b2
1	0.0000	cd
2	0.2093	a ad
2	0.2059	d2 ac
2	0.2030	ab bd
2	0.2004	d ac
2	0.1993	e2 ac
2	0.1951	e ac
2	0.1949	d2 ab
2	0.1936	b2 ab
2	0.1935	ac be
2	0.1894	d ab
2	0.1891	a2 d2

```
       2       0.1890    ac cd
       2       0.1882    e2 ab
       2       0.1849    a d2
       2       0.1841    d2 ae
       2       0.1840    e ab
       2       0.1837    d a2

                            Regression/selection=R^2

                               The REG Procedure
                                 Model: MODEL1
                           Dependent Variable: RGSyield

                             R-Square Selection Method

Number in
  Model      R-Square    Variables in Model

       2       0.1836    ac ad
       2       0.1828    ab ac
       2       0.1828    a2 e2
-------------------------------------------------------------------------------
       3       0.2932    b2 ab ad
       3       0.2789    d d2 ac
       3       0.2744    b b2 ab
       3       0.2695    b b2 ac
       3       0.2679    d d2 ab
       3       0.2594    d a2 d2
       3       0.2579    a d d2
       3       0.2571    d d2 ae
       3       0.2497    b a2 b2
       3       0.2485    a b b2
       3       0.2476    b b2 ae
       3       0.2464    ab bd be
       3       0.2459    b ab ad
       3       0.2451    c ac ad
       3       0.2432    d d2 ad
       3       0.2421    a ad de
       3       0.2411    ab ad bd
       3       0.2408    b2 ab be
       3       0.2379    c2 ac ad
       3       0.2375    a e2 ad
-------------------------------------------------------------------------------
       4       0.3791    c e ac ce
       4       0.3716    c e ab ce
       4       0.3674    c e2 ac ce
       4       0.3659    c e a2 ce
       4       0.3616    a c e ce
       4       0.3599    c e2 ab ce
       4       0.3582    c2 e2 ac ce
       4       0.3568    c a2 e2 ce
       4       0.3523    e c2 ac ce
       4       0.3499    a c e2 ce
       4       0.3481    e2 ac ad ce
       4       0.3471    c2 e2 ab ce
       4       0.3456    a2 c2 e2 ce
       4       0.3415    b b2 ab ad
       4       0.3399    e c2 ab ce
       4       0.3371    a c2 e2 ce
       4       0.3369    c e ae ce
       4       0.3364    e a2 c2 ce

                            Regression/selection=R^2

                               The REG Procedure
                                 Model: MODEL1
                           Dependent Variable: RGSyield

                             R-Square Selection Method

Number in
  Model      R-Square    Variables in Model

       4       0.3354    b2 ab ad be
       4       0.3337    e ac ad ce
-------------------------------------------------------------------------------
       5       0.4493    c e ac ad ce
       5       0.4376    c e2 ac ad ce
       5       0.4356    c2 e2 ac ad ce
       5       0.4331    e c2 ac ad ce
       5       0.4305    e d2 ac cd ce
```

```
       5       0.4265    d2 ac cd ce de
       5       0.4215    c e ac ae ce
       5       0.4213    e d2 ab cd ce
       5       0.4212    d2 e2 ac cd ce
       5       0.4209    a c e ad ce
       5       0.4174    d2 ab cd ce de
       5       0.4156    c d2 ac ce de
       5       0.4148    e a2 d2 cd ce
       5       0.4145    c e ab bd ce
       5       0.4140    c e d2 ac ce
       5       0.4129    d2 e2 ab cd ce
       5       0.4113    a e d2 cd ce
       5       0.4104    a2 d2 cd ce de
       5       0.4092    a c e2 ad ce
       5       0.4090    a2 d2 e2 cd ce
-------------------------------------------------------------------------------
       6       0.5061    c e ab ad bc ce
       6       0.5047    c e b2 ab ad ce
       6       0.4944    c e2 ab ad bc ce
       6       0.4906    c b2 e2 ab ad ce
       6       0.4870    c d e d2 ac ce
       6       0.4859    b c e b2 ab ce
       6       0.4822    c e2 ab bc be ce
       6       0.4801    b c e ab bc ce
       6       0.4796    c b2 e2 ab be ce
       6       0.4794    c d e d2 ab ce
       6       0.4778    c e ab ae bc ce
       6       0.4775    b c e b2 ac ce
       6       0.4764    b2 c2 e2 ab ad ce
       6       0.4764    c e b2 ab ae ce
       6       0.4757    e d2 ac ae cd ce
       6       0.4750    c b2 ab ad be ce
       6       0.4730    c e b2 ab be ce
       6       0.4726    c e ab bc be ce
       6       0.4717    b c e ac bc ce

                          Regression/selection=R^2

                              The REG Procedure
                                Model: MODEL1
                           Dependent Variable: RGSyield

                            R-Square Selection Method

Number in
  Model      R-Square    Variables in Model

       6       0.4717    d e d2 ac cd ce
-------------------------------------------------------------------------------
       7       0.5757    b c e b2 ab bc ce
       7       0.5673    b c e b2 ac bc ce
       7       0.5573    c e2 ab ad bc be ce
       7       0.5567    b c b2 e2 ab bc ce
       7       0.5556    c b2 e2 ab ad be ce
       7       0.5530    b c e b2 ab ad ce
       7       0.5519    c e b2 ab ad be ce
       7       0.5510    b c e a2 b2 bc ce
       7       0.5501    c e ab ad bc be ce
       7       0.5498    a b c e b2 bc ce
       7       0.5483    b c b2 e2 ac bc ce
       7       0.5477    b c e b2 ac ad ce
       7       0.5472    b c e ab ad bc ce
       7       0.5419    b c e ac ad bc ce
       7       0.5383    c e2 ac ad bc be ce
       7       0.5382    b e b2 d2 ab cd ce
       7       0.5360    b b2 d2 ab cd ce de
       7       0.5356    c b2 ab ad bc be ce
       7       0.5355    b c e2 ab ad bc ce
       7       0.5354    c b2 e2 ac ad be ce
-------------------------------------------------------------------------------
       8       0.6428    b c e b2 ab ad bc ce
       8       0.6375    b c e b2 ac ad bc ce
       8       0.6237    b c b2 e2 ab ad bc ce
       8       0.6184    b c b2 e2 ac ad bc ce
       8       0.6166    b c e b2 ab ae bc ce
       8       0.6156    b c e b2 ab bc bd ce
       8       0.6146    b c b2 d2 ab bc ce de
       8       0.6109    b c e b2 d2 ab bc ce
       8       0.6096    b c e b2 ac ae bc ce
       8       0.6091    a b c e b2 ad bc ce
       8       0.6072    b c e b2 ac bc bd ce
```

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        8       0.6062    b c b2 d2 ac bc ce de
        8       0.6050    b c d e b2 ab bc ce
        8       0.6031    b e b2 d2 ab bc cd ce
        8       0.6025    b c e b2 d2 ac bc ce
        8       0.6025    b c d e b2 d2 ab ce
        8       0.6024    b c e b2 ab bc ce de
        8       0.6018    b b2 d2 ab bc cd ce de
        8       0.6016    b c e b2 ab bc cd ce
        8       0.5983    b e b2 d2 ac bc cd ce

                           Regression/selection=R^2

                              The REG Procedure
                                Model: MODEL1
                           Dependent Variable: RGSyield

                             R-Square Selection Method

Number in
  Model      R-Square    Variables in Model

        9       0.6923    b c d e b2 d2 ab bc ce
        9       0.6838    b c d e b2 d2 ac bc ce
        9       0.6754    b c e b2 ab ac ad bc ce
        9       0.6663    a b c d e b2 d2 bc ce
        9       0.6660    b c d b2 d2 e2 ab bc ce
        9       0.6645    b c d e a2 b2 d2 bc ce
        9       0.6612    b c d b2 d2 ab bc ce de
        9       0.6576    b c d b2 d2 e2 ac bc ce
        9       0.6565    b c e b2 ab ae bc bd ce
        9       0.6564    b c b2 e2 ab ac ad bc ce
        9       0.6549    b c e b2 e2 ab ad bc ce
        9       0.6528    b c d b2 d2 ac bc ce de
        9       0.6528    b c e b2 ab ad bc be ce
        9       0.6518    b c e b2 d2 ab ae bc ce
        9       0.6515    b c d e b2 d2 ad bc ce
        9       0.6507    b c e a2 b2 ab ad bc ce
        9       0.6502    b c b2 e2 ab ad bc be ce
        9       0.6500    b c b2 d2 ab ae bc ce de
        9       0.6499    b c e b2 ac ad ae bc ce
        9       0.6496    b c e b2 e2 ac ad bc ce
-------------------------------------------------------------------------------
       10       0.7331    b c d e b2 d2 ab ae bc ce
       10       0.7300    b c d e b2 d2 ab ad bc ce
       10       0.7268    b c d e b2 d2 ac ad bc ce
       10       0.7262    b c d e b2 d2 ac ae bc ce
       10       0.7178    b c d e b2 d2 ab bc bd ce
       10       0.7168    a b c d e b2 d2 ad bc ce
       10       0.7094    b c d e b2 d2 ac bc bd ce
       10       0.7084    b c e b2 ab ac ad ae bc ce
       10       0.7062    a b c d e b2 d2 ab bc ce
       10       0.7038    b c d b2 d2 e2 ab ad bc ce
       10       0.7022    b c d e b2 d2 ab bc be ce
       10       0.7013    b c d e b2 d2 e2 ab bc ce
       10       0.7005    b c d b2 d2 e2 ac ad bc ce
       10       0.6990    b c d b2 d2 ab ad bc ce de
       10       0.6980    b c e b2 ab ac ad bc cd ce
       10       0.6969    a b c d e b2 d2 ac bc ce
       10       0.6969    a b c d e b2 d2 ae bc ce
       10       0.6965    b c e b2 ab ac ad bc ce de
       10       0.6963    b c d b2 d2 e2 ab ae bc ce
       10       0.6962    b b2 d2 ab ac ad bc cd ce de
-------------------------------------------------------------------------------
       11       0.7825    b c d e b2 d2 ab ac ad bc ce

                           Regression/selection=R^2

                              The REG Procedure
                                Model: MODEL1
                           Dependent Variable: RGSyield

                             R-Square Selection Method

Number in
  Model      R-Square    Variables in Model

       11       0.7632    b c d e b2 d2 ab ac ae bc ce
       11       0.7586    b c d e b2 d2 ab ae bc bd ce
       11       0.7567    a b c d e b2 d2 ab ad bc ce
       11       0.7563    b c d b2 d2 e2 ab ac ad bc ce
       11       0.7556    b c d e b2 d2 ab ad bc bd ce
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      11       0.7523    b c d e b2 d2 ac ad bc bd ce
      11       0.7517    b c d e b2 d2 ac ae bc bd ce
      11       0.7515    b c d b2 d2 ab ac ad bc ce de
      11       0.7474    a b c d e b2 d2 ac ad bc ce
      11       0.7474    a b c d e b2 d2 ad ae bc ce
      11       0.7447    b d e b2 d2 ab ac ad bc cd ce
      11       0.7431    b c d e b2 d2 ab ae bc be ce
      11       0.7424    a b c d e b2 d2 ad bc bd ce
      11       0.7422    b c d e b2 d2 e2 ab ae bc ce
      11       0.7400    b c d e b2 d2 ab ad bc be ce
      11       0.7391    b c d e b2 d2 e2 ab ad bc ce
      11       0.7381    b c d e b2 d2 ab ad ae bc ce
      11       0.7374    b c d e a2 b2 d2 ab ae bc ce
      11       0.7373    b c d e a2 b2 d2 ab ad bc ce
-------------------------------------------------------------------------------
      12       0.8081    b c d e b2 d2 ab ac ad bc bd ce
      12       0.8007    b c d e b2 d2 ab ac ad ae bc ce
      12       0.7925    b c d e b2 d2 ab ac ad bc be ce
      12       0.7916    b c d e b2 d2 e2 ab ac ad bc ce
      12       0.7887    b c d e b2 d2 ab ac ae bc bd ce
      12       0.7873    a b c d e b2 d2 ab ac ad bc ce
      12       0.7873    a b c d e b2 d2 ab ad ae bc ce
      12       0.7861    b c d e a2 b2 d2 ab ac ad bc ce
      12       0.7855    b c d e b2 d2 ab ac ad bc cd ce
      12       0.7843    b c d b2 d2 e2 ab ac ad bc be ce
      12       0.7837    b c d e b2 d2 ab ac ad bc ce de
      12       0.7834    b c d e b2 c2 d2 ab ac ad bc ce
      12       0.7828    b c d b2 d2 e2 ab ac ad bc ce de
      12       0.7823    a b c d e b2 d2 ab ad bc bd ce
      12       0.7818    b c d b2 d2 e2 ab ac ad bc bd ce
      12       0.7779    a b c d e b2 d2 ac ad ae bc ce
      12       0.7770    b c d b2 d2 ab ac ad bc bd ce de
      12       0.7751    b c d b2 d2 ab ac ad bc be ce de
      12       0.7731    b c d e b2 d2 ab ac ae bc be ce
      12       0.7729    a b c d e b2 d2 ac ad bc bd ce
-------------------------------------------------------------------------------
      13       0.8263    b c d e b2 d2 ab ac ad ae bc bd ce
      13       0.8180    b c d e b2 d2 ab ac ad bc bd be ce

                          Regression/selection=R^2

                              The REG Procedure
                                Model: MODEL1
                          Dependent Variable: RGSyield

                            R-Square Selection Method

Number in
  Model      R-Square    Variables in Model

      13       0.8178    a b c d e b2 d2 ab ac ad ae bc ce
      13       0.8171    b c d e b2 d2 e2 ab ac ad bc bd ce
      13       0.8128    a b c d e b2 d2 ab ac ad bc bd ce
      13       0.8128    a b c d e b2 d2 ab ad ae bc bd ce
      13       0.8116    b c d e a2 b2 d2 ab ac ad bc bd ce
      13       0.8111    b c d e b2 d2 ab ac ad bc bd cd ce
      13       0.8107    b c d e b2 d2 ab ac ad ae bc be ce
      13       0.8098    b c d b2 d2 e2 ab ac ad bc bd be ce
      13       0.8098    b c d e b2 d2 e2 ab ac ad ae bc ce
      13       0.8093    b c d e b2 d2 ab ac ad bc bd ce de
      13       0.8090    b c d e b2 c2 d2 ab ac ad bc bd ce
      13       0.8083    b c d b2 d2 e2 ab ac ad bc bd ce de
      13       0.8059    b c d e a2 b2 d2 ab ac ad ae bc ce
      13       0.8037    b c d e b2 d2 ab ac ad ae bc cd ce
      13       0.8035    a b c d e b2 d2 ac ad ae bc bd ce
      13       0.8019    b c d e b2 d2 ab ac ad ae bc ce de
      13       0.8016    b c d e b2 c2 d2 ab ac ad ae bc ce
      13       0.8016    b c d e b2 d2 e2 ab ac ad bc be ce
-------------------------------------------------------------------------------
      14       0.8434    a b c d e b2 d2 ab ac ad ae bc bd ce
      14       0.8362    b c d e b2 d2 ab ac ad ae bc bd be ce
      14       0.8353    b c d e b2 d2 e2 ab ac ad ae bc bd ce
      14       0.8314    b c d e a2 b2 d2 ab ac ad ae bc bd ce
      14       0.8293    b c d e b2 d2 ab ac ad ae bc bd cd ce
      14       0.8278    a b c d e b2 d2 ab ac ad ae bc be ce
      14       0.8275    b c d e b2 d2 ab ac ad ae bc bd ce de
      14       0.8272    b c d e b2 c2 d2 ab ac ad ae bc bd ce
      14       0.8271    b c d e b2 d2 e2 ab ac ad bc bd be ce
      14       0.8269    a b c d e b2 d2 e2 ab ac ad ae bc ce
      14       0.8238    b c d b2 d2 e2 ab ac ad bc bd be ce de
      14       0.8228    a b c d e b2 d2 ab ac ad bc bd be ce
```

```
      14       0.8228    a b c d e b2 d2 ab ad ae bc bd be ce
      14       0.8228    b c d b2 d2 e2 ab ac ad ae bc bd ce de
      14       0.8219    a b c d e b2 d2 e2 ab ac ad bc bd ce
      14       0.8219    a b c d e b2 d2 e2 ab ad ae bc bd ce
      14       0.8216    b c d b2 d2 e2 ab ac ad ae bc bd be ce
      14       0.8216    b c d e a2 b2 d2 ab ac ad bc bd be ce
      14       0.8213    b c d e a2 b2 d2 e2 ab ac ad bc bd ce
      14       0.8210    b c d e b2 d2 ab ac ad bc bd be cd ce
-------------------------------------------------------------------------------
      15       0.8534    a b c d e b2 d2 ab ac ad ae bc bd be ce
      15       0.8525    a b c d e b2 d2 e2 ab ac ad ae bc bd ce
      15       0.8464    a b c d e b2 d2 ab ac ad ae bc bd cd ce

                          Regression/selection=R^2

                             The REG Procedure
                               Model: MODEL1
                           Dependent Variable: RGSyield

                           R-Square Selection Method

Number in
  Model      R-Square    Variables in Model

      15       0.8454    a b c d e a2 b2 d2 ab ac ad ae bc bd ce
      15       0.8453    b c d e b2 d2 e2 ab ac ad ae bc bd be ce
      15       0.8446    a b c d e b2 d2 ab ac ad ae bc bd ce de
      15       0.8443    a b c d e b2 c2 d2 ab ac ad ae bc bd ce
      15       0.8414    b c d e a2 b2 d2 ab ac ad ae bc bd be ce
      15       0.8413    b c d e a2 b2 d2 e2 ab ac ad ae bc bd ce
      15       0.8394    b c d b2 d2 e2 ab ac ad ae bc bd be ce de
      15       0.8393    b c d e b2 d2 ab ac ad ae bc bd be cd ce
      15       0.8384    b c d e b2 d2 e2 ab ac ad ae bc bd cd ce
      15       0.8381    a b c d b2 d2 e2 ab ac ad ae bc bd ce de
      15       0.8375    b c d e b2 d2 ab ac ad ae bc bd be ce de
      15       0.8371    b c d e b2 c2 d2 ab ac ad ae bc bd be ce
      15       0.8369    a b c d e b2 d2 e2 ab ac ad ae bc be ce
      15       0.8366    b c d e b2 c2 d2 e2 ab ac ad ae bc bd ce
      15       0.8366    b c d e b2 d2 e2 ab ac ad ae bc bd ce de
      15       0.8357    a b c d b2 d2 e2 ab ac ad ae bc bd be ce
      15       0.8345    b c d e a2 b2 d2 ab ac ad ae bc bd cd ce
-------------------------------------------------------------------------------
      16       0.8624    a b c d e b2 d2 e2 ab ac ad ae bc bd be ce
      16       0.8564    a b c d e b2 d2 ab ac ad ae bc bd be cd ce
      16       0.8555    a b c d e b2 d2 e2 ab ac ad ae bc bd cd ce
      16       0.8553    a b c d e a2 b2 d2 ab ac ad ae bc bd be ce
      16       0.8553    a b c d b2 d2 e2 ab ac ad ae bc bd be ce de
      16       0.8549    a b c d e a2 b2 d2 e2 ab ac ad ae bc bd ce
      16       0.8546    a b c d e b2 d2 ab ac ad ae bc bd be ce de
      16       0.8543    a b c d e b2 c2 d2 ab ac ad ae bc bd be ce
      16       0.8537    a b c d e b2 c2 d2 e2 ab ac ad ae bc bd ce
      16       0.8537    a b c d e b2 d2 e2 ab ac ad ae bc bd ce de
      16       0.8512    b c d e a2 b2 d2 e2 ab ac ad ae bc bd be ce
      16       0.8484    a b c d e a2 b2 d2 ab ac ad ae bc bd cd ce
      16       0.8483    b c d e b2 d2 e2 ab ac ad ae bc bd be cd ce
      16       0.8476    a b c d e b2 d2 ab ac ad ae bc bd cd ce de
      16       0.8473    a b c d e b2 c2 d2 ab ac ad ae bc bd cd ce
      16       0.8466    a b c d e a2 b2 d2 ab ac ad ae bc bd ce de
      16       0.8466    b c d e b2 c2 d2 e2 ab ac ad ae bc bd be ce
      16       0.8465    b c d e b2 d2 e2 ab ac ad ae bc bd be ce de
      16       0.8464    a b c d e a2 b2 c2 d2 ab ac ad ae bc bd ce
      16       0.8455    a b c d e b2 c2 d2 ab ac ad ae bc bd ce de
-------------------------------------------------------------------------------
      17       0.8654    a b c d e b2 d2 e2 ab ac ad ae bc bd be cd ce
      17       0.8649    a b c d e a2 b2 d2 e2 ab ac ad ae bc bd be ce
      17       0.8637    a b c d e b2 c2 d2 e2 ab ac ad ae bc bd be ce
      17       0.8637    a b c d e b2 d2 e2 ab ac ad ae bc bd be ce de

                          Regression/selection=R^2

                             The REG Procedure
                               Model: MODEL1
                           Dependent Variable: RGSyield

                           R-Square Selection Method

Number in
  Model      R-Square    Variables in Model

      17       0.8584    a b c d e a2 b2 d2 ab ac ad ae bc bd be cd ce
      17       0.8583    a b c d b2 d2 e2 ab ac ad ae bc bd be cd ce de
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```
      17       0.8580    a b c d a2 b2 d2 e2 ab ac ad ae bc bd be ce de
      17       0.8579    a b c d e a2 b2 d2 e2 ab ac ad ae bc bd cd ce
      17       0.8576    a b c d e b2 d2 ab ac ad ae bc bd be cd ce de
      17       0.8573    a b c d e b2 c2 d2 ab ac ad ae bc bd be cd ce
      17       0.8567    a b c d e b2 c2 d2 e2 ab ac ad ae bc bd cd ce
      17       0.8567    a b c d e b2 d2 e2 ab ac ad ae bc bd cd ce de
      17       0.8567    a b c d b2 c2 d2 e2 ab ac ad ae bc bd be ce de
      17       0.8566    a b c d e a2 b2 d2 ab ac ad ae bc bd be ce de
      17       0.8564    a b c d e a2 b2 c2 d2 ab ac ad ae bc bd be ce
      17       0.8564    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd ce
      17       0.8561    a b c d e a2 b2 d2 e2 ab ac ad ae bc bd ce de
      17       0.8555    a b c d e b2 c2 d2 ab ac ad ae bc bd be ce de
      17       0.8549    a b c d e b2 c2 d2 e2 ab ac ad ae bc bd ce de
      17       0.8542    b c d e a2 b2 d2 e2 ab ac ad ae bc bd be cd ce
-------------------------------------------------------------------------------
      18       0.8679    a b c d e a2 b2 d2 e2 ab ac ad ae bc bd be cd ce
      18       0.8667    a b c d e b2 c2 d2 e2 ab ac ad ae bc bd be cd ce
      18       0.8667    a b c d e b2 d2 e2 ab ac ad ae bc bd be cd ce de
      18       0.8663    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be ce
      18       0.8661    a b c d e a2 b2 d2 e2 ab ac ad ae bc bd be ce de
      18       0.8649    a b c d e b2 c2 d2 e2 ab ac ad ae bc bd be ce de
      18       0.8610    a b c d a2 b2 d2 e2 ab ac ad ae bc bd be cd ce de
      18       0.8597    a b c d b2 c2 d2 e2 ab ac ad ae bc bd be cd ce de
      18       0.8596    a b c d a2 b2 c2 d2 e2 ab ac ad ae bc bd be ce de
      18       0.8596    a b c d e a2 b2 d2 ab ac ad ae bc bd be cd ce de
      18       0.8594    a b c d e a2 b2 c2 d2 ab ac ad ae bc bd be cd ce
      18       0.8594    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd cd ce
      18       0.8592    a b c d e a2 b2 d2 e2 ab ac ad ae bc bd cd ce de
      18       0.8585    a b c d e b2 c2 d2 ab ac ad ae bc bd be cd ce de
      18       0.8579    a b c d e b2 c2 d2 e2 ab ac ad ae bc bd cd ce de
      18       0.8576    a b c d e a2 b2 c2 d2 ab ac ad ae bc bd be ce de
      18       0.8576    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd ce de
      18       0.8558    b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce
      18       0.8555    b c d e a2 b2 d2 e2 ab ac ad ae bc bd be cd ce de
      18       0.8540    b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be ce de
-------------------------------------------------------------------------------
      19       0.8694    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce
      19       0.8691    a b c d e a2 b2 d2 e2 ab ac ad ae bc bd be cd ce de
      19       0.8679    a b c d e b2 c2 d2 e2 ab ac ad ae bc bd be cd ce de
      19       0.8676    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be ce de
      19       0.8627    a b c d a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce de

                          Regression/selection=R^2

                              The REG Procedure
                                Model: MODEL1
                          Dependent Variable: RGSyield

                            R-Square Selection Method

Number in
  Model      R-Square    Variables in Model

      19       0.8606    a b c d e a2 b2 c2 d2 ab ac ad ae bc bd be cd ce de
      19       0.8606    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd cd ce de
      19       0.8570    b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce de
      19       0.8471    a b d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce de
      19       0.8450    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc be cd ce de
      19       0.8400    a b c d e a2 b2 c2 d2 e2 ab ac ad bc bd be cd ce de
      19       0.8400    a b c d e a2 b2 c2 d2 e2 ab ad ae bc bd be cd ce de
      19       0.8307    a b c d e a2 b2 c2 d2 e2 ac ad ae bc bd be cd ce de
      19       0.8201    a b c d e a2 b2 c2 d2 e2 ab ac ae bc bd be cd ce de
      19       0.7958    a b c e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce de
      19       0.7896    a b c d e a2 b2 c2 e2 ab ac ad ae bc bd be cd ce de
      19       0.7808    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bd be cd ce de
      19       0.7806    a c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce de
      19       0.7732    a b c d e a2 c2 d2 e2 ab ac ad ae bc bd be cd ce de
      19       0.6904    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd de
-------------------------------------------------------------------------------
      20       0.8706    a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce de
```

```
data a;
input a b c d e RGSyield;
cards;
120   12    40.0  20    7           18
```

```
60    9     47.5  22    6           16.5
120   12    40.0  20    5           15.8
120   12    25.0  20    5           16.7
180   15    32.5  18    6           20.1
60    15    47.5  18    6           14.3
60    9     32.5  18    6           16.6
120   6     40.0  20    5           14.7
120   12    40.0  16    5           14.9
180   9     32.5  22    6           14.5
120   12    40.0  20    5           15.7
180   15    47.5  18    4           19.3
120   12    40.0  20    5           15.9
60    15    47.5  22    4           13.9
60    15    32.5  18    4           14.7
120   18    40.0  20    5           14.5
0     12    40.0  20    5           14.4
120   12    40.0  20    5           17.1
120   12    40.0  20    5           18.5
120   12    40.0  20    5           17.5
180   15    32.5  22    4           14.4
180   9     32.5  18    4           14.6
180   9     47.5  18    6           16.2
120   12    40.0  20    3           17.2
240   12    40.0  20    5           20.1
180   15    47.5  22    6           14.9
60    9     47.5  18    4           16.3
120   12    40.0  24    5           14.7
60    9     32.5  22    4           14.0
60    15    32.5  22    6           18.0
120   12    40.0  20    5           16.9
120   12    55.0  20    5           17.6
180   9     47.5  22    4           17.9
;
data a;
set a;
a2=a*a;
b2=b*b;
c2=c*c;
d2=d*d;
e2=e*e;
ab=a*b;
ac=a*c;
ad=a*d;
ae=a*e;
bc=b*c;
bd=b*d;
be=b*e;
cd=c*d;
ce=c*e;
de=d*e;
run;

title 'RSreg';
proc rsreg data=a;
model RGSyield=a b c d e /nocode press predict  lackfit  ;
ridge min  radius = 0 to 2 by .1;
run;
```

```
option nodate nonumber;
title 'Regression';
proc reg data=a;
model RGSyield=a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce de;
run;
quit;
title 'Regression/selection=stepwise';
proc reg data=a;
model RGSyield=a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce
de/selection=stepwise;
run;
quit;
title 'Regression/selection=R^2';
proc reg data=a;
model RGSyield=a b c d e a2 b2 c2 d2 e2 ab ac ad ae bc bd be cd ce
de/selection=rsquare;
run;
quit;
```

Stepwise Regression Analysis for Red Dog, Germ, and Shorts – Step 2

```
Regression/b c d b2 d2 ab ac ad bc ce de

                The REG Procedure
                  Model: MODEL1
            Dependent Variable: RGSyield

Number of Observations Read          33
Number of Observations Used          33

                Analysis of Variance

                                Sum of           Mean
Source                   DF    Squares         Square    F Value    Pr > F

Model                    11   75.32920        6.84811       5.77    0.0003
Error                    21   24.91262        1.18632
Corrected Total          32  100.24182

          Root MSE              1.08918    R-Square     0.7515
          Dependent Mean       16.25455    Adj R-Sq     0.6213
          Coeff Var             6.70078

                Parameter Estimates

                          Parameter       Standard
Variable     DF            Estimate          Error    t Value    Pr > |t|

Intercept     1          -59.72917       20.95802      -2.85      0.0096
b             1            2.55949        0.73889       3.46      0.0023
c             1            0.85390        0.22782       3.75      0.0012
d             1            4.39230        2.00811       2.19      0.0402
b2            1           -0.06507        0.02192      -2.97      0.0073
d2            1           -0.13391        0.04932      -2.71      0.0130
ab            1            0.00310        0.00143       2.17      0.0416
ac            1            0.00114     0.00054256       2.11      0.0475
ad            1           -0.00349        0.00127      -2.76      0.0118
bc            1           -0.03333        0.01210      -2.75      0.0119
ce            1           -0.11353        0.03206      -3.54      0.0019
de            1            0.24158        0.06465       3.74      0.0012
```

```
data a;
input a b c d e RGSyield;
cards;
120   12    40.0  20    7           18
60    9     47.5  22    6           16.5
120   12    40.0  20    5           15.8
120   12    25.0  20    5           16.7
180   15    32.5  18    6           20.1
60    15    47.5  18    6           14.3
60    9     32.5  18    6           16.6
120   6     40.0  20    5           14.7
120   12    40.0  16    5           14.9
180   9     32.5  22    6           14.5
120   12    40.0  20    5           15.7
180   15    47.5  18    4           19.3
120   12    40.0  20    5           15.9
60    15    47.5  22    4           13.9
60    15    32.5  18    4           14.7
120   18    40.0  20    5           14.5
0     12    40.0  20    5           14.4
```

```
120   12    40.0  20    5           17.1
120   12    40.0  20    5           18.5
120   12    40.0  20    5           17.5
180   15    32.5  22    4           14.4
180   9     32.5  18    4           14.6
180   9     47.5  18    6           16.2
120   12    40.0  20    3           17.2
240   12    40.0  20    5           20.1
180   15    47.5  22    6           14.9
60    9     47.5  18    4           16.3
120   12    40.0  24    5           14.7
60    9     32.5  22    4           14.0
60    15    32.5  22    6           18.0
120   12    40.0  20    5           16.9
120   12    55.0  20    5           17.6
180   9     47.5  22    4           17.9
;
data a;
set a;
a2=a*a;
b2=b*b;
c2=c*c;
d2=d*d;
e2=e*e;
ab=a*b;
ac=a*c;
ad=a*d;
ae=a*e;
bc=b*c;
bd=b*d;
be=b*e;
cd=c*d;
ce=c*e;
de=d*e;
run;

title 'Regression/b c d b2 d2 ab ac ad bc ce de';
proc reg data=a;
model RGSyield=  b c d b2 d2 ab ac ad bc ce de;
run;
quit;
```