FREE AND BOUND PHENOLIC ACID CONTENTS OF MICHIGAN-GROWN WHEAT VARIETIES AND RETENTION DURING COOKIE BAKING

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ABSTRACT

FREE AND BOUND PHENOLIC ACID CONTENTS OF MICHIGAN-GROWN WHEAT VARIETIES AND RETENTION DURING COOKIE BAKING

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Plant phenolic compounds have seen increased interest within the field of cereal science in the recent decade, indicated by the ongoing research in their functional and health properties. The objectives of this thesis were (1) to quantify the total phenolic acid and ferulic acid contents of selected Michigan grown wheat varieties harvested in 2013 and 2014, and (2) to compare differences in post-processing antioxidant retention and baking properties between cookies fortified with flour using four different antioxidant formulations (20% bran, 4.4% insoluble arabinoxylan powder, ferulic acid powder, or 20% digested bran with ferulic acid powder). The total phenolic acid contents ranged from 3.6 to 6.8 mg GAE/g for the 2013 crop year, and 4.0 to 6.0 mg GAE/g for the 2014 crop year. The total ferulic acid contents ranged from 272 ± 12 μg/g to 412 ± 1 μg/g for the 2013 crop year, and from 316 ± 36 μg/g to 467 ± 34 μg/g for the 2014 crop year. The ratios of the averaged total phenolic acid contents in the milled fractions of bran, shorts, and flour for all varieties were 5:4:1. For the baking analysis, the 20% bran blend showed the least amount of ferulic acid degradation after baking among the flour blends studied, with a 90% retention rate, and is the recommended form for ferulic acid fortification.

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TABLE OF CONTENTS

LIST OF TABLES vii

LIST OF FIGURES ix

KEY TO ABBREVIATIONS xi

CHAPTER 1
INTRODUCTION 1

CHAPTER 2
LITERATURE REVIEW 4
2.1. Overview of Ferulic Acid 4
2.2. Health Benefits of Ferulic Acid 7
2.3. Factors Affecting Antioxidant Contents in Different Wheat Varieties 9
2.4. Bioavailability of Phenolic Acids 10
2.5. Extraction of Phenolic Acids 11
2.6. Effects of Storage and Processing on Phenolic Acid Contents 14
2.7. Commercial Feasibility 15

ChHAPTER 3
MATERIALS AND METHODS 17
3.1.Materials 17
3.1.1. Wheat kernel Samples 17
3.1.2. Chemicals 20
3.2. Methods 20
3.2.1. Milling of Wheat Fractions and Basic Wheat Characterization 20
3.2.2.Experimental Outline of Antioxidant Quantification Analysis 22
3.2.3. Extraction of Phenolic Acids by Alkaline Digestion 24
3.2.4. Extraction of Ferulic Acid by Enzymatic Digestion 25
3.2.5. Quantification of Wheat Flour Total Phenolic Contents by Colorimetric Analysis 28
3.2.6. Quantification of Wheat Flour Ferulic Acid Content by Reverse-Phase High-Performance Liquid Chromatography 28
3.2.7. Ferulic Acid Stability during Cookie Baking 30
3.3. Data analysis: ANOVA 33

CHAPTER 4
RESULTS AND DISCUSSION 34
4.1. Moisture, Ash, and Protein Contents and Soundness of Wheat Samples 34
4.2. Total Phenolic Content (TPC) of Wheat Lines 39
4.2.1. Total Phenolic Content (TPC) of Whole Ground Flour 39
4.2.2.Total Phenolic Content of Milling Fractions of Wheat Varieties 48

4.3. Ferulic Acid Content of Whole Ground Flour 52
4.4. Pearson Correlations of the Phenolic and Ferulic Acid Contents of Studied Varieties..... 58
4.5. Effect of Crop Year on Ferulic Acid Content of Whole Ground Flour Samples our 60
4.6. Baking Quality of Cookies after Fortification with Free and Bound Forms of Ferulic Acid 64
4.6.1. Visual Quality of Cookies Baked from Flour Fortified with Free and Bound Forms of Ferulic Acid 64
4.6.2. Effect of Ferulic Acid Source on Cookie Moisture Contents 66
4.6.3. Effect of Ferulic Acid Source on Cookie Color 68
4.6.4. Cookie Diameter and Height 71
4.6.5. Cookie Hardness 74
4.6.6. Changes in Total Ferulic Acid Contents after Baking..... 74

CHAPTER 5
CONCLUSIONS AND FUTURE RECOMMENDATIONS 80

APPENDICES 82
APPENDIX A. Supplementary Data for Wheat Phenolic Quantification and Cookie Baking 83
APPENDIX B. Viability of Enzymatic Digestion of Wheat Flour with Feruloyl Esterase 87
APPENDIX C. Statistical Analysis Raw Data. 92
APPENDIX D. Colorimetric Analysis Raw Data 129

LITERATURE CITED..... 135

LIST OF TABLES

Table 1. Name Listing of MSU Soft Wheat Varieties from 2013 and 2014 Crop Years Selected for Testing 18

Table 2. Feruloyl Esterase (FAE) and Xylanase (Xyl) Enzyme Composition, and Heat Usage, in Treatment of Whole Ground Wheat Flour Samples to Extract Ferulic Acids 27

Table 3.a. Moisture, Ash, and Protein Contents of Whole Ground Flour from Michigan Wheat Varieties from the 2013 Harvest (n=3) 35

Table 3.b. Moisture, Ash, and Protein Contents of Whole Ground Flour from Michigan Wheat Varieties from the 2014 Harvest (n=3) 37

Table 4. Ferulic Acid Contents of Select 2013 MSU Wheat Breeding Varieties 53

Table 5. Ferulic Acid Contents of Select 2014 MSU Wheat Breeding Varieties 55

Table 6. Select Environmental Conditions of Growing Locations in East-Central Michigan from the harvesting and planting seasons of 2013 to 2014 (MAWN, 2012,2013,2014) 63

Table 7. Moisture Contents of Cookies Baked From Different Ambassador Wheat Flour Blends 67

Table 8. Color Analysis of Ferulic Acid-Fortified Cookies made from Ambassador flour 69

Table 9. Physical Qualities of Cookies Baked from Different Blends of Ambassador Wheat Flour Fortified with Identical Amounts of Ferulic Acid (n=3) 73

Table 10. Preparation of ferulic acid standard solution for RP-HPLC analysis 83

Table 11. Pearson Product-Moment Correlation Coefficient between Different Parameters from Cookie Baking 84

Table 12. Significant Differences of Total Populations measured using One way ANOVA with Fisher Comparison. Populations with $p<0.05$ were deemed significantly different 85

Table 13.1. Raw Data for 2013 Total Ferulic Acid Content 93

Table 13.2. Raw Data for 2013 Bound Ferulic Acid Content 96

Table 13.3. Raw Data for 2013 Free Ferulic Acid Content 99

Table 13.4. Raw Data for 2014 Total Ferulic Acid Content 102

Table 13.5. Raw Data for 2014 Bound Ferulic Acid Content 105

Table 13.6. Raw Data for 2014 Free Ferulic Acid Content 108

Table 13.7. Raw Data for 2013 Total Phenolic Acid Content 111
Table 13.8. Raw Data for 2013 Bound Phenolic Content 114
Table 13.9. Raw Data for 2013 Free Phenolic Content..... 117
Table 13.10. Raw Data for 2014 Total Phenolic Content 120
Table 13.11. Raw Data for 2014 Bound Phenolic Content 123
Table 13.12. Raw Data for 2014 Free Phenolic Content 126
Table 14.1. Phenolic Acid Contents of Selected 2013 MSU Wheat Breeding Varieties 129
Table 14.2. Phenolic Acid Contents of Selected 2014 MSU Wheat Breeding Varieties 131
Table 14.3. Total Phenolic Acid Contents of 2013 Wheat Fractions 132
Table 14.4. Total Phenolic Acid Contents of 2014 Wheat Fractions 134

LIST OF FIGURES

Figure 1. Structure of Ferulic Acid (Srinivasan et al., 2001)..... 6

Figure 2. Resonance Stabilization of Ferulic Acid (Srinivasan et al., 2001)..... 6

Figure 3. Procedural Outline for Total Phenolic Content and Ferulic Acid Quantification of Wheat Samples. 23

Figure 4.a. Total Phenolic Contents of Wheat Varieties from the 2013 MSU Wheat Breeding Program. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13. 42

Figure 4.b. Bound Phenolic Contents of Varieties from the 2013 MSU Wheat Breeding Program. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13. Bound phenolic content is obtained by subtracting the free from the total phenolic contents. 43

Figure 4.c. Free Phenolic Contents of Lines from the 2013 MSU Wheat Breeding Program. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13. 44

Figure 5.a. Total Phenolic Contents of 2014 MSU Wheat Breeding Varieties. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13.. 45

Figure 5.b. Bound Phenolic Contents of 2014 MSU Wheat Breeding varieties. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13. Bound phenolic content is obtained by subtracting the free from the total phenolic contents. 46

Figure 5.c. Free Phenolic Contents of 2014 MSU Wheat Breeding varieties. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13. 47

Figure 6. Total Phenolic Contents of Milled Wheat Fractions from Selected Michigan wheat varieties harvested in 2013. Samples were analyzed in triplicate and results are reported as mean values with one standard deviation. 50

Figure 7. Total Phenolic Contents of Milled Wheat Fractions from Selected Michigan Wheat Varieties harvested in 2014. Samples were analyzed in triplicate and reported as mean values with one standard deviation. 51

Figure 8. Representative Visual Appearance of Cookies baked using Different Flour Blends Consisting of white flour and: (a) No additions, (b) 20% bran, (c) 4.4% arabinoxylan, (d) Free FA powder, and (e) Digested 20% bran with free FA powder 65

Figure 9. Total Ferulic Acid Content of Baked Cookie Samples made from Various White Flour Blends. Bars not sharing the same letter are significantly different from each other ($p<0.05$). The blue line is an indication of the initially fortified level measured in the cookie dough of the treatments before baking, excluding the original control flour. The ferulic acid content of the control cookie without fortification was included for reference.
..... 78

Figure 10. Percent of Ferulic Acid Retained in Cookies after Baking Cookie Doughs made from Four Different White Flour Blends of Wheat Variety Ambassador. 79

Figure 11. UV-Visible Spectrum of Ferulic Acid Extracted from Michigan Soft Wheat Whole Ground Flour (Ambassador). 86

Figure 12. Ferulic Content of Wheat Samples Treated with Constant Xylanase at 20 U and increasing amounts of Feruloyl Esterase (see Table 2, without pre-heating treatment). 88

Figure 13. Ferulic Acid Content of Wheat Samples Treated with Constant FE at 20 U and Increasing Concentrations of Xyl (Table 2, with heating). 90

Figure 14. Ferulic Acid Content of Wheat Samples Treated With Constant Levels of Xyl at 20 U, and Increasing Additions of FE, With Heat Pre-Treatment. 91

KEY TO ABBREVIATIONS

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

DDGS: distiller's dried grain with solubles

DPPH: 2,2-diphenyl-1-picrylhydrazyl

FA: Ferulic acid

FE: Feruloyl esterase

GA: Gallic acid

GAE: Gallic Acid Equivalent

HPLC: High Performance Liquid Chromatography

MAWN: Michigan Automated Weather Network

ORACS: Oxygen radical absorbance capacity

PPO: Polyphenol oxidase

TPC: Total Phenolic Contents

TFA: Trifluoroacetic acid

PC: Phenolic Contents

Xyl: Xylanase

CHAPTER 1

INTRODUCTION

Cereal grains contain a wide variety of antioxidants, including phenolic acids, flavonoids, and condensed tannins (Dykes and Rooney, 2007). Phenolic compounds are not as widely studied as other antioxidants such as vitamins A, C, and E, but they are highly abundant in nature, and are essential components in many plant structures. In the past decade there has been increasing interest in phenolic compounds due to their potential benefits to human health. Studies on these compounds have indicated protective effects when ingested in sufficient quantities (Dillard and German, 2000).

Bound phenolic acids, such as ferulic acid, have been found to have greater bioavailability than free ferulic acids (Rondini et al., 2004). The majority of ferulic acid in wheat flour is in its bound form, but many of the existing antioxidant fortification studies using ferulic acid used free soluble ferulic acid powder, and did not mention potential differences in processing properties between bound vs free ferulic acids (Ou et al., 2005; Koh and Ng 2008). During food processing such as baking, bound ferulic acid in wheat flour may be more stable and experience less loss than its free form, being less available for reaction with the other food ingredients. This means that it may not be applicable to use the results of experiments utilizing free ferulic acid powder to interpret effects on naturally-occurring bound ferulic acid existing in plant products. Free ferulic acid is also more accessible to yeast and other microorganisms, which may metabolize it and break it down to inactive components, decreasing the overall antioxidant capability of a flour (Huang et al., 1993). It is therefore important to conduct

experiments that isolate bound and free ferulic acids and observe their individual effects on human nutrition and food processing.

There are new food product development opportunities in promoting higher levels of such intrinsic antioxidants for nutritional value and health benefits, although current U.S. regulations do not permit labeling claims for any antioxidants nor any phenolic acids. To be eligible to claim the health benefits reported by nutritional and medical studies (Sultana et al., 2005; Kroon et al., 1997), the bioactive antioxidants require standardization of effective dosages. Many by-products of food processing are rich in phenolic compounds. Examples include wheat bran and straw, olive oil mill waste, and distiller's dried grain with solubles (DDGS) from biofuel production (Ezeji and Blaschek, 2008). These products may serve as sources of abundant raw material for commercial phenolic acid production and fortification of foods.

Quantifying the antioxidant capacity and ferulic acid content of current wheat varieties and future lines can lead to understanding of the diversity of wheat antioxidants. Michigan primarily produces soft red winter (SRW) and soft white winter (SWW) wheats, and there are research programs focusing on the development of new soft wheat lines. Soft white winter wheat is the main focus of research in this thesis. The antioxidant and ferulic acid contents of Michigan-grown soft wheat have not been determined, and survey efforts of antioxidant capacity and ferulic acid content among SRW and SWW wheat samples from the MSU breeding program is warranted. Screening for wheat varieties with the genetic potential for high antioxidant content yields would provide the Michigan wheat farming and milling sectors the

ability to produce new food ingredients and products containing higher antioxidant contents as adaptation for shifting consumer preferences and market demands.

The minimum physiologically effective dose is yet to be agreed upon. In cooperation with ongoing current research on the health benefits of phenolic acids, the phenolic acid contents of Michigan wheat varieties should be surveyed to assist in determining the amounts of phenolic acids naturally present in wheat for future reference. Quantification of Michigan wheat antioxidant content will assist wheat breeding programs in selecting for varieties with different antioxidant yields. The objectives of this study were to:

1) Determine the contents of bound and free phenolic and ferulic acids of selected Michigan-grown soft wheat varieties.

2) Compare the differences in post-processing antioxidant retention of bound ferulic acid and free ferulic acid, and the baking qualities of cookies made with different flour formulations fortified with the same level of antioxidants (bound ferulic acid or free ferulic acid).

CHAPTER 2

LITERATURE REVIEW

2.1. Overview of Ferulic Acid

Phenolic acids are phenolcarboxylic acids with aromatic components, containing at least one phenolic ring and a carboxylic functional group. They are commonly found within many types of legumes, cereals, and fruits (Shahidi and Naczk, 2003), and are the most common type of antioxidants found in wheat. Phenolic acids in wheat are most abundant in the aleurone layer, with lesser amounts found in the germ and endosperm (Yu, 2008). They perform many important functions in plant metabolism, and are often produced in response to changes in environmental factors such as light and temperature changes (Solecka and Kacperska, 2003).

Ferulic acid belongs to the family of hydroxycinnamic acids alongside *p*-coumaric and caffeic acids, and it is the most commonly found phenolic acid in wheat (Figure 1). Other types of phenolic compounds are present in small quantities, such as vanillic, caffeic, salicylic, and *p*-coumaric acids (Adom et al., 2003). The antioxidant ability of wheat phenolic acids is due to their phenol groups, with the most basic form consisting of a hydrocarbon ring bonded to a hydroxyl group. The basic antioxidant mechanism involves the donation of hydrogen atoms to quench free radicals, and has been shown to be effective in the scavenging of superoxide anion radicals and inhibition of peroxidation in lipids (Srinivasan et al., 2007). Ferulic acid is similar in structure to its precursor caffeic acid, both of which are important structural constituents in lignin (Boerjan, 2003), the difference being the methoxy group replacing the second hydroxyl group in ferulic acid (Figure 1). The possession of a phenolic nucleus and conjugated side chain

allows ferulic acid to donate hydrogen atoms to free radicals and form a resonance-stabilized phenoxy radical (Srinisavan et al., 2007, Figure 2). Ferulic acid has also been shown to have synergistic interactions with other antioxidants such as α-tocopherol, β-carotene, and ascorbic acid (Trombino et al., 2004).

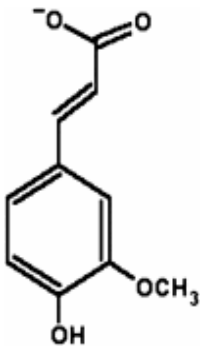

Figure 1. Structure of Ferulic Acid (Srinivasan et al., 2001).

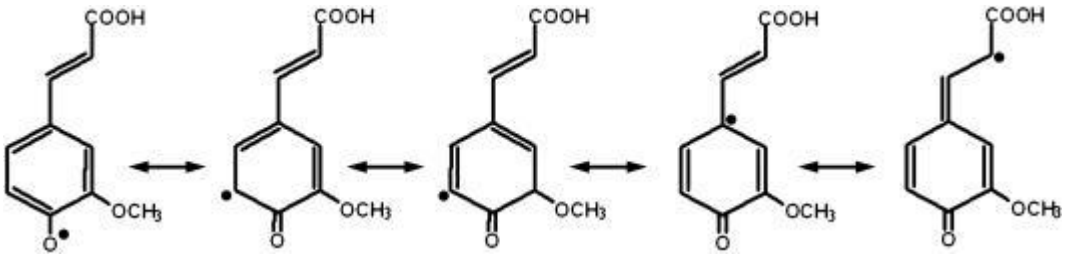

Figure 2. Resonance Stabilization of Ferulic Acid (Srinivasan et al., 2001).

In wheat, ferulic acid exists in both freely soluble form and bound insoluble form (hereafter referred to as bound form), with the bound form composing the majority of the total phenolic acid content (Yu, 2008). The bound form of ferulic acid is commonly attached through ester linkages to arabinoxylan, a hemicellulose found in the primary and secondary cell walls of grass plants. It can also be found linked to other plant structures in lesser amounts, such as to cellulose, lignin, and proteins (McCartney et al., 2005; Pussayanawin et al., 1988). The presence of phenolic acids within the structural units of plants are linked to anti-microbial and anti-fungal activities, possibly serving as a protective agent against surface infection (Sarma and Singh, 2003). Similar anti-microbial effects attributed to phenolic compounds were reported from the addition of millet seed coat extracts into millet flour, indicating the possible use for phenolic acids as natural food preservatives, and demonstrating their versatile functional properties (Viswanath et al., 2009).

2.2. Health Benefits of Ferulic Acid

The average daily intake of ferulic acid has been estimated to be between 150-250 mg in a healthy and varied diet (Zhao and Moghadasian, 2008). Studies have been performed on the potential health benefits of phenolic acids, including ferulic acids from wheat. Ferulic acid has been shown to reduce the amount of free radicals produced from oxidative stress in rats with different induced illnesses. From the study performed by Balasubashni and colleagues, rats with induced diabetes were fed high dosages of ferulic acids at 40 mg/kg, and up to a threefold decrease was seen in the presence of lipid peroxidation products in the liver, such as

hydroperoxides and free fatty acids (Balasubashni et al., 2004). Sultana and colleagues simulated neuron damage from Alzheimer's disease by treating rat brain tissue cultures with amyloid β-peptide, and found the addition of ferulic acid ethyl ester activated protective genes and enzymes in rat brain tissue cultures, with decreases in reactive oxygen species accumulation, protein oxidation and lipid peroxidation (Sultana et al., 2005). Mice whose diets were supplemented with ferulic acid in drinking water showed decreased levels of brain inflammation and oxidative stress induced by injections of β-amyloid peptides (Yan et al., 2001). Ferulic acid has also been shown to have a dose-dependent inhibitory effect on T47D human breast cancer cells and induced apoptosis. It was found that the same phenol rings and carbon side chain groups responsible for anti-oxidative properties also played important roles in cancer cell suppression (Kampa et al., 2004). Ferulic acid also displays potential for being a colon anticarcinogen as part of a high fiber diet (Kroon et al., 1997). Furthermore, ferulic acid has shown anti-inflammatory, anti-thrombotic activities, and protection against cardiovascular illnesses, while having low toxicity and being easily absorbed (Ou and Kwok, 2004). Increase in fiber intake has been known to decrease the risk of colon cancer, and sustained daily consumption of wheat bran has been shown to decrease the growth of polyp cells in colorectal cancer patients (Alberts et al., 1990). These studies focused on increased fiber consumption as the cause for the observed changes, but the abundance of phenolic acids in natural food fiber could also be taken into consideration as one of the possible mechanisms behind colon cancer reduction.

2.3. Factors Affecting Antioxidant Contents in Different Wheat Varieties

There are both genetic and environmental factors determining wheat antioxidant levels. An *in vitro* study of phenolic exudation by the young roots of different wheat varieties around the world showed large differences in antioxidant levels. The amount of measured *trans*-ferulic acid varied from 1.6 to 23.4 μg/L in water/agar solutions, and the amount of *cis*-ferulic acid ranged from 0.33 to 12.7 μg/L (Wu et al., 2001). Adom and colleagues (2003) analyzed 11 diverse wheat varieties including hard, soft, and durum wheat varieties from Cornell University Small Grains Breeding and Genetics Program, and the total phenolic contents ranged from 1.2 mg/g to 1.4 mg/g in gallic acid equivalents (GAE) per gram of wheat sample; their HPLC analysis of total ferulic acid content revealed results between 249.9 μg/g (red spring durum) and 513.4 μg/g (white spring durum). The soft white winter variety of Caledonia had 340.1 μg/g of total ferulic acid content.

In addition to grain genotype and growing location, processing conditions and part of the grain sampled also affects phenolic content levels (Adom and Liu, 2002; Adom et al., 2003, 2005). Mpofu et al. (2006) did not find grain color (red vs. white) to significantly affect the expression of antioxidant genes in Canadian Western wheat varieties, and did not find growing temperature and rainfall to affect it, either. They concluded that the location of planting was more significant than weather conditions in their interaction with wheat genotypes for antioxidant contents (Mpofu et al., 2006). Liyana-Pathirana and Shahidi (2006) compared the phenolic contents of soft and hard Eastern Canadian wheat, and found the whole ground flour of the soft wheat yielded 46.9 mg/g in ferulic acid equivalents using ferulic acid standards, and

that of the hard wheat yielded 40.6 mg/g. Their report had the highest measured ferulic acid contents, which were several times larger than the findings from other published studies. Mpofu et al. (2006) analyzed 6 varieties of hard spring wheat from western Canada, and the ferulic acid contents as measured by HPLC ranged from 371 μg/g to 441 μg/g. Their study showed that the range of ferulic acid contents in different wheat varieties is location-dependent.

2.4. Bioavailability of Phenolic Acids

Phenolic acids have generally been shown to be bioavailable in *in vivo* tests, but with differences upon ingestion of the soluble (free) and insoluble (bound) forms. Bourne and Rice-Evans (1998) found free ferulic acid and feruloyl glucuronide excreted in human urine to be 11-25% of the amount ingested. Rats that were fed bran which contained bound ferulic acid had an increased duration of ferulic acid in the bloodstream, and a decreased amount excreted through the urine (Rondini et al., 2004; Adam et al., 2002; Kroon et al., 1997). Rondini observed increased plasma antioxidant activity in the bran group alongside lower ferulic acid excretion, and reported this as bound ferulic acid having higher bioavailability than free ferulic acid (Rondini et al., 2004). The overall consensus is that bound ferulic acids, which are attached to arabinoxylans and other indigestible fibers, were prevented from being fully absorbed in the small intestine, leading to reduced bioavailability (Anson et al., 2009). It has been theorized that after passing the small intestine, bound ferulic acids could be released from the bran via bacterial fermentation within the large intestine with a slower absorption rate (Ou and Kwok, 2004). This means that although bound ferulic acid is absorbed more slowly than free ferulic

acid, it may still show similar metabolized quantities over a longer period of time, although this has yet to be studied. The lower intestinal flora was proposed to be a source of bacterial esterase and xylanase activity, and thereby responsible for the separation of ferulic acid from the soluble and insoluble fibers (Kroon et al., 1997).

2.5. Extraction of Phenolic Acids

In industrial production, it is important to maximize the extraction yield of phenolic compounds from their raw materials. There is currently no unified procedure for extraction and processing of cereal antioxidants, and different published papers have favored different methods with varying results with situational strengths and weaknesses, such as differences in reagent and solvent toxicity, cost efficiency, rate of extraction, and feasibility in large scale commercial operations (Yu, 2008). It is beneficial to conduct more research in newer extraction methods to discover potential new advantages, and to identify weak points in older extraction methods. The food industry can benefit from the understanding of reasons for different antioxidant extraction conditions to maximize phenolic acid production specific to their production conditions.

Polar protic solvents, such as methanol and ethanol, are favored for phenolic acid extraction due to high solvent solubility (Daneshfar et al., 2008), which appears to be due to hydrogen bonding between hydroxyl groups of the solvent and oxygen atoms in the phenolic acids (Thomas et al., 2012). Sultana et al. (2009) found aqueous solvent mixtures to be able to extract more phenolic acids than their pure counterpart solvents, and shaking produced higher

total phenolic yields than using reflux. Zhou and Yu (2004) extracted ground wheat samples with different aqueous solvents: 50% acetone, 70% absolute ethanol, or 70% methanol. Extraction with 50% acetone yielded the highest measured antioxidant readings, and TPC extraction with 70% methanol produced the lowest measurements, having about half of the reading values obtained with 50% acetone. Others have reported 80% methanol as capable of extracting the greatest amount of ferulic acid (Yu, 2008). Yu (2008) reported the most effect solvent to be a 7:7:6 mixture of methanol, acetone and water. Inglett et al., (2009) found 50% ethanol to be the most effective extraction solvent. Pure 100% ethanol remains the most cost-effective extraction solvent for large scale commercial processes (Yu et al., 2002).

The amount of bound phenolic compounds present in wheat is higher than that of their free forms in all wheat varieties tested (Liyana-Pathyrana and Shahidi, 2006, Adom et al., 2003), and detection requires the breakdown of their attachment to the plant cell wall materials. Ferulic acid is attached by an ester linkage to arabinoxylan, a hemicellulose common in members of the grass family. Alkaline or heated acidic digestion is the common method of releasing almost all bound phenolic acids from wheat, which is cost effective to perform in large batches, and also completes within a relatively short time. Kim and colleagues (2006) found wheat bran samples digested by alkaline digestion showed more antioxidant activity than those that underwent acidic digestion. Enzymatic digestion of cereal grains for the release of specific phenolic acids has not been widely researched. Initial method development was performed by Faulds and Williamson (1995), in which feruloyl esterase obtained from *Aspergillus niger* was applied to destarched wheat bran at different concentrations. Many phenolic acids such as

ferulic acid have better water solubility at higher temperatures (Noubigh et al, 2007), indicating a preference for extraction methods with heat-stable reagents.

There are factors to take into consideration in the identification and quantification of phenolic acids, which may hinder the experiment if not addressed. During solvent extraction, stages of washing and reconstitution to purify the sample (Yu, 2008) can lead to inevitable minor losses, requiring the usage of either internal or external standards to determine the recovery rate. Due to the spectral similarities and retention times of some phenolic acids and their smaller quantities in comparison to ferulic acid, it is possible for one large peak reading of ferulic acid to obscure other smaller peaks in HPLC analysis (Yu, 2008). Changes in starch viscosity at different pH levels may result in incomplete antioxidant extraction due to decreased mobility, and changes in pH itself can destabilize some antioxidant structures. The specificity varies widely between extraction methods. Older colorimetric analytic methods could not be used to identify any specific compounds, while modern methods such as HPLC can identify nearly all the components of a sample reading. Enzymatic extraction differs from other extraction methods by its ability to isolate specific compounds in the extraction stage instead of the analysis stage, minimizing interference from other miscellaneous materials which can be released by alkaline extraction.

2.6. Effects of Storage and Processing on Phenolic Acids Content

Long term storage of wheat kernels in unfavorable conditions leads to deterioration in germination ability and decrease in antioxidant content, aided by increased cell membrane permeability and breakdown in structural proteins such as glutenins and gliadins, as shown in accelerated aging tests (Galleschi et al., 2002). Milled wheat kernels release more antioxidants, and whole wheat kernels are able to preserve more antioxidant content than milled fractions over time, so the unprocessed kernel form is recommended for long term storage (Cheng et al., 2006). Free phenolic contents of various cereals increase after sprouting, and subsequently decrease following baking, leading to overall loss in total phenolic contents. This suggests that bound phenolic acids may be protected against thermal damage during processing (Alvarez-Jubete et al., 2010).

Wheat kernels lost about 15% of their phenolic content after 12 hours of soaking in water, while germination for 48 hours decreased the phenolic content of various cereals products by 39-44% (Ramadan et al., 2012). Gélinas and McKinnon (2005) found that the baking of whole wheat bread broke down bound phenolic acid esters into their free forms. Extrusion has a positive effect on the release of phenolic acids; Zielinski et al. (2001) measured a 200-300% increase in measurable free phenolic content in various cereal flours upon extrusion. This was hypothesized to be due to the conversion from bound to free forms of phenolic acids during hydrothermal processing, possibly from the destruction of cell wall material. Ferulic acid remained the highest measured phenolic compound after extrusion (Zielinski et al., 2001). In baking tests conducted by Moore and colleagues on pizza products, 48 hours of extra

fermentation time led to a 130% increase in free ferulic acid. An increase in baking temperature from 204 ^{o}C to 288^{o}C with constant baking time increased ferulic acid content by 82%, while an increase in baking time from 7 to 14 min with constant temperature increased ferulic acid content up to 60% (Moore et al., 2009). From these observations, differing cooking methods could directly impact the quantity of phenolic acids in foods.

2.7. Commercial Feasibility

Due to the development of social media in the last decade, the spread and change in popular opinion on health and diet has accelerated in pace. The recent diet trends, such as the ketogenic diet (Freeman et al., 2007), in the popular health and fitness culture have encouraged drastic reduction of carbohydrates in daily food intake,. In 2011, the USDA changed their nutrition guidelines from the Food Guide Pyramid to a plate-shaped pie chart called Myplate, which significantly reduced the size and visual emphasis of carbohydrate groups in daily meals (Bush, 2012). From these recent changes, it is important to address popular perceptions of high-starch foods, to optimize their impacts on cereal consumption and wheat production. Promotion of the potential health benefits of wheat consumption and active participation in influencing and directing popular culture would be a logical step in improving the public perception of wheat-based food products.

Phenolic acids have potential within the supplement industry as candidates for product development, comparable to novelty items such as grape extract and krill oil. These products are ingredients derived from waste product reclamation, which improves the cost efficiency of

raw material production and processing. For wheat, large amounts of antioxidant-rich primary processing by-products such as straw, chaff, bran, and shorts are discarded in cereal harvesting and milling. Recovery of these materials can create new sources of revenue through the commercial production and sale of extractable phenolic acids (Balasundram et al., 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Wheat kernel samples

Selected soft wheat varieties were provided by the Michigan State University Wheat Breeding Program located in East Lansing, MI, USA. Fifty-seven varieties from the 2013 harvest and 39 varieties from the 2014 harvest were tested. Of these two harvest years, 34 varieties were grown in both years (Table 1).

Table 1. Name Listing of MSU Soft Wheat Varieties from 2013 and 2014 Crop Years Selected for Testing

Crop Year 2013		Crop Year 2014	
Variety Number	**Variety Name**	**Variety Number**	**Variety Name**
1	MSU Line F1014		
2	MSU Line F1026R	2	MSU Line F1026R
3	MSU Line F1012		
4	MSU Line F1003R		
5	MSU Line F1029	5	MSU Line F1029
6	MSU Line F1047	6	MSU Line F1047
7	MSU Line F1032R		
8	MSU Line F1027	8	MSU Line F1027
9	MSU Line F1049	9	MSU Line F1049
10	MSU Line F1051		
11	MSU Line F1050		
12	MSU Line F1048	12	MSU Line F1048
13	MSU Line F2031	13	MSU Line F2031
14	MSU Line F2033	14	MSU Line F2033
15	MSU Line F2034	15	MSU Line F2034
16	MSU Line F2032		
17	MSU Line F2037	17	MSU Line F2037
18	MSU Line F2035		
19	MSU Line F2041		
20	MSU Line F2036		
21	MSU Line F2038	21	MSU Line F2038
22	MSU Line F2040		
23	MSU Line F2042	23	MSU Line F2042
24	MSU Line F2039	24	MSU Line F2039
25	MSU Line F2001R		
26	MSU Line F2006		
27	MSU Line F2005	27	MSU Line F2005
28	MSU Line F2015	28	MSU Line F2015
29	MSU Line F2009	29	MSU Line F2009
30	MSU Line F2012	30	MSU Line F2012
31	MSU Line F2008		
32	MSU Line F2011		
33	MSU Line F2014R	33	MSU Line F2014R
34	MSU Line F2003	34	MSU Line F2003

Table 1 (cont'd)

35	MSU Line F2004		
36	MSU Line F2002	36	MSU Line F2002
37	MSU Line F2028R	37	MSU Line F2028R
38	MSU Line F2020	38	MSU Line F2020
39	MSU Line F2024R	39	MSU Line F2024R
40	MSU Line F2018	40	MSU Line F2018
41	MSU Line F2019	41	MSU Line F2019
42	MSU Line F2016	42	MSU Line F2016
43	MSU Line F2022	43	MSU Line F2022
44	MSU Line F2025R		
45	MSU Line F2021	45	MSU Line F2021
46	MSU Line F2030	46	MSU Line F2030
47	MSU Line F2029R	47	MSU Line F2029R
48	MSU Line F2027R		
49	Aubrey	49	Aubrey
50	Hopewell		
51	Ambassador	51	Ambassador
52	Jupiter	52	Jupiter
53	Red Ruby		
54	VA09W-188WS		
55	Cayuga		
56	VA09W-192WS		
57	Caledonia		
		58	F2010
		59	Unnamed 1
		60	Unnamed 2
		61	Unnamed 3
		62	F0013R

3.1.2. Chemicals

Chemicals used in the experiments were as follows: Folin-Ciocalteu reagent, anhydrous sodium hydroxide, 12N hydrochloric acid, anhydrous sodium carbonate, diethyl ether, and hexane (all from Sigma-Aldrich Co., LLC, St. Louis, MO); commercially produced ferulic acid powder (Avantor Performance Materials, Center Valley, PA); feruloyl esterase (*Clostridium thermocellum*), xylanase, and insoluble arabinoxylan power (all from Megazyme International Ireland Ltd., Wicklow, Ireland).

3.2. Methods

3.2.1. Milling of Wheat Fractions and Basic Wheat Characterization

The following milling procedure was performed according to the AACC International Approved Methods, AACCI Method 26-31.01.

The moisture contents of the wheat kernel samples were measured by a Motomco 919 Moisture Meter (Motomco Ltd., Madison, WI). Kernels (250 g) were weighed and placed within the dump cell container of the moisture meter, and the moisture content reading was given by an electric current meter. Temperature adjustment was performed using a calibration table and an electric thermometer. The kernels of the entire sample (5 kg) were tempered to 14.5% moisture content overnight before milling.

For producing whole ground flour, Approximately 150 grams of kernels of each wheat variety with as-is moisture was ground by a Perten KT-3100 laboratory mill (Perten Instruments, Springfield, IL) to pass through a 0.5 mm sieve to prepare whole meal powder. The whole meal powder was sealed in airtight Ziploc bags and stored at 4°C until analyses.

The moisture, protein, and ash contents of the whole ground wheat flour at 14% moisture basis of the Michigan wheat samples (Table 1) were measured using a Bruker Multi-Purpose FT-NIR (Near Infrared) Spectrometer (Bruker Corp., Billerica, MA) (AACCI Method 39-00.01).

Milling of the wheat kernels into bran, shorts and straight grade flour was performed using a Buhler Automatic Mill MLU-202 (Buhler Inc., Austin, TX). The samples (5 kg) were tempered to 14.5% moisture content overnight (in two steps as indicated above), and then milled at a feed rate of 55-70 g/min. The kernels were stored in room temperature, and the milled flour and wheat fractions were put into Ziploc bags and placed into cold storage at about 4^{o}C.

The Falling Number test was performed using a Perten Falling Number System (Perten Instruments, Springfield, IL) according to AACCI Method 56-81.03 to determine whether the wheat samples were sprouted or sound. Each flour sample was adjusted to 7 g and 14% moisture basis and mixed with 25 ml of distilled water in a stoppered test tube. The tubes were shaken by a Perten Shakematic shaker, placed within a receptacle containing boiling water, and mixed for 60 seconds. Sensor rods were dropped and timed until they reached the bottom of

the tubes. The total time in seconds required for the rod to drop through the wheat slurry to the bottom of the tube is defined as the Falling Number value for that particular sample.

3.2.2. Experimental Outline of Antioxidant Quantification Analysis

A flow diagram for the quantification of total phenolic contents and ferulic acid in the studied wheat samples is depicted in Figure 3, indicating the order of the experiments. Due to time constraints and hierarchy of experiment importance, only colorimetric analysis was performed on the milled wheat fractions, whereas colorimetric analysis and RP-HPLC analysis were performed on the whole ground flour.

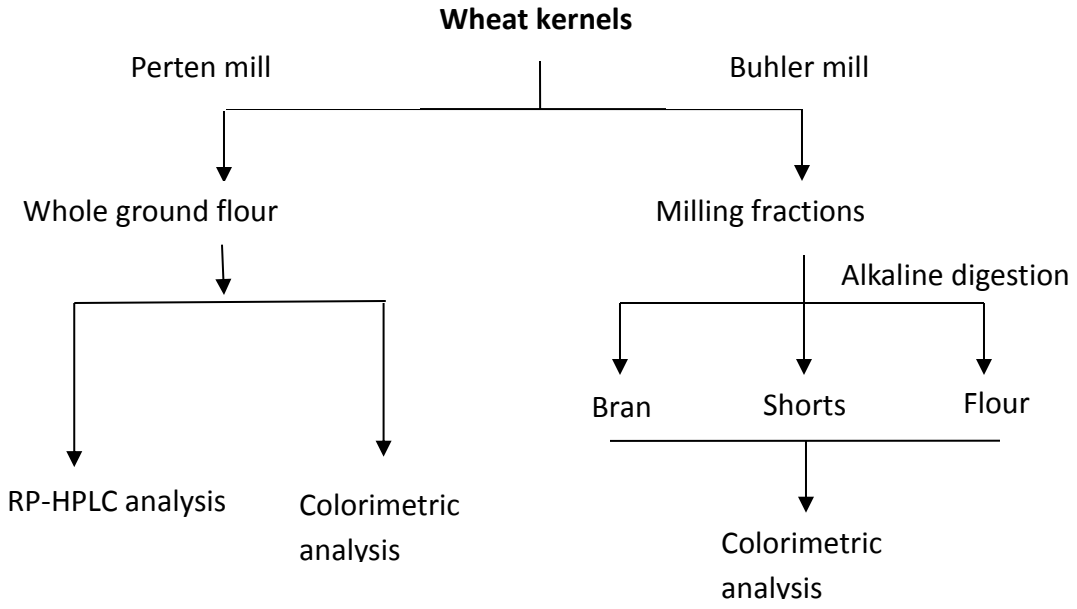

Figure 3. Procedural Outline for Total Phenolic Content and Ferulic Acid Quantification of Wheat Samples.

3.2.3. Extraction of Phenolic Acids by Alkaline Digestion

Extraction of free phenolic acids was performed on the whole ground wheat flour of the Michigan wheat varieties (Table 1). The samples of the ground whole flour and wheat fractions were randomly selected from cold storage and warmed to room temperature for approximately 2 hours, and 0.500 ± 0.004 g of a sample was placed in a 50 ml polypropylene centrifuge tube, filled with 40 ml of 80% methanol/water mixture, nitrogen purged, and shaken for 4 hours at room temperature at 75 rpm under darkness. The shaker used was a New Brunswick Scientific Gyrotory Shaker, model G2 (New Brunswick Scientific Co., Inc., Edison, NJ). After 4 hours, the samples were centrifuged at 2500 x g for 10 minutes at 23^{o}C using a Beckman J2 21m centrifuge (Beckman Coulter Inc., Pasadena, CA). The resulting supernatant containing the free ferulic acids was collected.

The alkaline extraction procedure for the bound phenolic acids from the wheat flour samples was performed on whole ground wheat flour, and on the milled bran, shorts and flour fractions of the Michigan wheat varieties (Table 1). The method follows the summary given by Adom and colleagues (Adom et al., 2003). Flour samples at approximately 150 g each bag were randomly selected from cold storage and warmed to room temperature for 2 hours. For each sample, 0.500 ± 0.004 g was placed in a 50 ml polypropylene centrifuge tube. 20 ml of 2N NaOH solution (2N, 20 ml) were pipetted into each centrifuge tube followed immediately by 20 seconds of nitrogen flushing. The samples were rapidly mixed for 30 seconds by a Fisher Genie 2 Vortex (Fisher Scientific Inc., Waltham, MA) to ensure complete dispersion of the flour particles, and shaken for 4 hours at room temperature at 75 rpm using a New Brunswick

Scientific Gyrotory Shaker model G2 (New Brunswick Scientific Co., Inc., Edison, NJ). The shaker was covered with a box to avoid light exposure. After 4 hours, the samples were neutralized with 3.33 ml of 12N HCl, and centrifuged at 2500 x g for 10 minutes at 23^{o}C using a Beckman J2 21m centrifuge (Beckman Coulter Inc., Pasadena, CA). The supernatant was collected and transferred into glass centrifuge tubes, washed once with 2:1 volume of hexane to remove free fatty acids, and extracted with 2:1 volume of ethyl acetate three times by vortexing and pipetting. The ethyl acetate was evaporated to dryness using a Rotovap Rotary Evaporator (Buchi Corp., New Castle, DE), and reconstituted with 10 ml of deionized water. This was immediately tested for bound phenolic acids (3.2.5) then discarded due to lack of storage space.

3.2.4. Extraction of Ferulic Acid by Enzymatic Digestion

The procedure for enzymatic extraction of ferulic acid from wheat flour samples was performed as outlined by Faulds and Williamson (1995). The material analyzed was whole ground flour from the variety Ambassador, which was chosen as a representative sample for method development and the experiments were performed in triplicate. For each replicate, whole ground flour from Ambassador (0.01 g) was weighed into a 15 ml microcentrifuge tube. Acetic acid/sodium acetate buffer (50 mM, pH 4.5, 1 ml) was pipetted into each tube. After vortexing for 20-30 seconds, the tubes were pre-heated on a Fisher Scientific Isotemp Heated Magnetic Stirrer/Hotplate (Fisher Scientific Inc., Waltham, MA) for 30 minutes at 100^{o}C, and were taken out and vortexed every 5 minutes using a Fisher Genie 2 vortex (Fisher Scientific Inc., Waltham, MA). The samples were cooled to room temperature and vortexed to disperse

again. Different quantities of feruloyl esterase and xylanase (Table 2) were pipetted into the sample tubes, and the samples were capped and immersed immediately afterwards into a Julabo SW22 Shaking Water Bath (Julabo USA Inc., Allentown, PA) for 3 hours at 50^{o}C and 200 rpm, with vortexing every 15 minutes during digestion. After the digestion was completed, the samples were centrifuged using a Fisher 235B microcentrifuge at 1000 x g (Fisher Scientific Inc., Waltham, MA). The supernatant containing ferulic acid was collected and analyzed immediately with no leftover storage. Colorimetric testing (3.2.5) and RP-HPLC analysis (3.2.6) were performed on each of the supernatants.

Table 2. Feruloyl Esterase[1] (FAE) and Xylanase[1] (Xyl) Enzyme Composition, and Heat Usage[2], in Treatment of Whole Ground Wheat Flour Samples to Extract Ferulic Acids

Treatment number	Enzyme composition	Temperature pre-treatment (100^{o}C)
1	No enzymes	No
2	No enzymes	Yes
3	2 μl FE; 20 μl Xyl	Yes
4	4 μl FE; 20 μl Xyl	Yes
5	5 μl FE; 20 μl Xyl	Yes
6	10 μl FE; 20 μl Xyl	Yes
7	15 μl FE; 20 μl Xyl	Yes
8	20 μl FE; 20 μl Xyl	Yes
9	No enzymes	Yes
10	20 μl FE; 2 μl Xyl	Yes
11	20 μl FE; 4 μl Xyl	Yes
12	20 μl FE; 5 μl Xyl	Yes
13	20 μl FE; 10 μl Xyl	Yes
14	20 μl FE; 15 μl Xyl	Yes
15	20 μl FE; 20 μl Xy	Yes

[1] *The concentration of ferulic acid esterase (FE) was 1.26 U/μl. The concentration of xylanase (Xyl) was 1.00 U/μl. U stands for activity unit, which is the amount of enzyme needed to digest 1 μmol of substrate/min.*

[2] *For details, see appendix B.*

3.2.5. Quantification of Wheat Flour Total Phenolic Contents by Colorimetric Analysis

The materials analyzed were the supernatants containing the free and bound phenolic acids (Sections 3.2.3 and 3.2.4.) from the whole ground flour, the combined phenolic acids from the milled wheat fractions (Section 3.2.1), and the enzymatically digested whole ground flour of variety Ambassador (Section 3.2.4), from the selected Michigan wheat varieties (Table 1). Folin-Ciocalteu reagent is a liquid mixture composed of phosphomolybdate and phosphotungstate. It is commonly used in colorimetric *in vitro* assays for the measurement of phenolic acids. This reagent measures the total reducing capacity of a sample, which usually uses a gallic acid standard, also known as Gallic Acid Equivalent (GAE), which was prepared for this study in 10, 20, 50, 100, and 200 mg/L concentrations in deionized water solutions. For this experiment, 100 μl of the bound and free phenolic extracts (Section 3.2.3) from the wheat flour, 100 μl of Folin-Ciocalteu reagent, and 1.5 ml of distilled water were pipetted into a disposable cuvette immediately in that order. After 8 minutes, 300 μl of 20% sodium carbonate solution were added, and the cuvette was immediately shaken and read for absorbance at 760 nm on a Spectronic Genesys 5 Spectrophotometer (Thermo Scientific Inc., Waltham, MA). Each sample was measured in triplicate.

3.2.6. Quantification of Wheat Flour Ferulic Acid Content by Reverse-Phase High-Performance Liquid Chromatography

The samples from the free and bound phenolic acid extractions (Section 3.2.4) were filtered sequentially through 100 μm then 45 μm syringe filters into 2 ml autosampling bottles.

The equipment used was an Alliance 2669 HPLC System with a diode array detector (Waters Corp., Milford, MA). The samples were injected with a built in autosampler and autoinjector set at 10 μl.

The mobile phase used two solvents: solvent A was 100% deionized water with 0.1% trifluoroacetic acid (TFA), and solvent B was 100% methanol with 0.1% TFA, with a flow rate of 0.8 ml/min and a gradient time of 45 minutes. The gradient program was as follows: 100% A to 70% A in 5 min, 70% A to 50% A in 15 min, 50% A to 5% A in 30min, and 5% A to 100% A in 3 min. Reconditioning was performed using a 100% methanol solution with 0.1% TFA for 45 minutes at the beginning of analysis and 10 minutes at the end of analysis. Peak absorbance was determined at 320 nm (Appendix A, Figure 11). The column used was a Phenomenex Luna® 5 μm C18 (2) 100 Å, LC, 250 x 4.6 mm Column (Phenomenex Inc., Torrance, CA). The peak area and retention time were recorded using the Alliance HPLC System Software (Waters Corp., Milford, MA). The maximum acceptable column pressure was 3500 psi, with normal column pressure fluctuating between 2000-2500 psi. Ferulic acid was used as the standard and prepared in concentrations of 10, 20, 50, and 100 μg ferulic acid/L deionized water (Appendix A, Table 10).

3.2.7. Ferulic Acid Stability during Cookie Baking

For flour preparation, the Ambassador variety of wheat was the sole variety used, and served as the representative variety for this experiment. Approximately 5 kg of kernels were tempered to 14.5% moisture and milled into white flour, shorts, and bran fractions following the method in section 3.2.1. For each of the different flour blends described below, approximately 100 g of sample were weighed, and then mixed in a plastic bottle in a rotating drum for 4 hours.

The cookie flour blends were: (1) white flour (control), (2) white flour with 0.0324% (w/w) free ferulic acid, (3) white flour with 4.4% (w/w) commercial insoluble wheat arabinoxylan powder, (4) white flour with 20% (w/w) bran, and (5) white flour with 20% (w/w) digested bran (existing phenolic acids removed) and 0.0324% (w/w) free ferulic acid.

For blend (5), milled bran fraction from Ambassador was first subjected to alkaline digestion with 2N NaOH for 4 hours to remove the existing ferulic acid. This treated bran was dried in an Isotemp drying oven (Fisher Scientific Inc., Waltham, MA) at 50^{o}C for 24 hours and reground into powder (0.5 mm sieve size) using a coffee grinder. Polyphenol oxidase activity is very high in bran (Okot-Kotber et al., 2001), and for this treatment the neutralized extraction solvent was preserved and dried together with the bran to retain the polyphenol oxidase in the dried bran fraction for observing the effects of enzymatic browning during baking.

The total ferulic acid content of the Ambassador wheat kernel was 324 μg ferulic acid/g whole ground flour, with free ferulic acid content at 19 μg ferulic acid/g whole ground flour. All of the flour blends (except for the control) had a targeted ferulic acid content of 324 μg ferulic acid/g flour, including the preexisting free ferulic acid content in the flour, except for blend (#5),

which removed all preexisting ferulic acids from the flour, with added 0.0324% (w/w) free ferulic acid powder to account for the natural free/bound ferulic acid ratio.

The prepared flour blends were used to bake micro wire-cut cookies according to the AACCI Method 10-54.01. The cookie ingredients used for one batch of cookies were: 12.8 g white sugar, 4 g brown sugar, 0.4 g nonfat dry milk powder, 0.5 g non-iodized salt, 0.4 g sodium bicarbonate, 16 g vegetable shortening, 8.8 ml water, 40 g flour (or flour blend) at 14% moisture basis, 0.6 g high fructose corn syrup, 0.2 g ammonium bicarbonate. Ammonium bicarbonate and fructose syrup were combined with the water to form the wet ingredients. The dry ingredients, except for the flour, were mixed with vegetable shortening for 3 minutes to form 34.1 g of creamed mass in a Hobart cake mixer (Hobart Corporation, Lansing, MI). The creamed mass and wet ingredients were mixed together in a National Mfg 100 g mixing bowl (National MFG Co., Lincoln, NE) for 1 minute, then flour was added and the dough was mixed for 30 seconds. The resulting dough was sheeted and cut on metal trays, then baked for 11 minutes at 400^{o}F in a National Mfg Rotary Baking Oven (National MFG Co., Lincoln, NE). Three replicates of each treatment were baked, with each replicate having two batches, and each batch producing two cookies.

The diameter and height of the cookies were measured with rulers and calipers, and the cookies were measured with two stacked on top of each other for each batch. Photos of the cookies were taken for visual comparison, and cookie color and darkening were quantitatively measured using a CR-410 Chroma Meter (Konica Minolta Sensing Americas Inc., Ramsey, NJ). Cookie hardness was tested using a TA-HDi Texture Analyzer (Microsystems Ltd., Hamilton, MA),

and followed the procedure from the American Institute of Baking and Texture Technologies (American Institute of Baking, Manhattan, KS). A TA-42 knife fixture was used to cut the cookie, and compression force was measured in Newtons. The test speed was 2.0 mm/second and the distance of cutting was 15.0 mm. Measurements were made from three cookies of each flour blend sample and data were averaged.

For the ferulic acid quantification, two baked cookies from each baking test for each blend sample were ground in a coffee grinder in 10-second intervals for a total of 30 seconds, followed by the alkaline digestion extraction and RP-HPLC analysis protocols previously described in sections 3.2.5 and 3.2.6. Samples were analyzed in triplicate.

3.3. Data Analysis: ANOVA

One-way ANOVA was used to determine the statistical significance of the bound and free phenolic contents and ferulic acid measurements, using Minitab Statistical Software (Minitab Inc., State College, PA). The α-level was set at 0.05. The main purpose of this analysis was to determine whether there would be any statistically similar groups within the wheat varieties, using Fisher pairwise comparisons (Appendix C, Table 13).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Moisture, Ash, and Protein Contents and Soundness of Wheat Samples

The whole ground flour of the wheat varieties harvested in 2013 and 2014 were tested for their moisture, ash, protein contents, and falling number. The whole ground flour was prepared as mentioned in 3.1.1. All wheat varieties displayed normal ranges of moisture, ash and protein contents that are typical of the values found in commercially used soft wheat flour. (Table 3 a, b). Most of the wheat varieties were sound, having Falling Number values above the 250 second threshold, indicating little to no pre-sprouting. (Table 3 a, b). There were slight increases for the 2014 year in protein content in many of the flour samples, but the overall differences between the two years was not significantly different when measured using paired t-test, with a p-value of 0.065.

Table 3.a. Moisture, Ash, and Protein Contents of Whole Ground Flour from Michigan Wheat Varieties from the 2013 Harvest (n=3)

Crop Year 2013				
Variety Number	**Moisture content (%)**	**Ash content (%)**	**Protein content (%)**	**Falling Number (s)**
1	10.43	1.34	10.29	333
2	10.61	1.48	10.22	368
3	10.00	1.21	8.98	316
4	11.72	1.53	11.61	375
5	11.29	1.19	9.92	312
6	11.74	1.58	9.67	354
7	11.08	1.53	9.67	342
8	11.14	1.17	9.51	361
9	11.26	1.48	9.41	412
10	11.54	1.39	9.99	375
11	10.91	1.25	9.92	391
12	11.40	1.53	8.94	343
13	11.14	1.50	9.74	346
14	10.70	1.27	10.71	329
15	10.40	1.20	11.18	335
16	10.78	1.55	10.25	381
17	10.74	1.32	11.45	191
18	10.22	1.31	10.86	323
19	10.41	1.42	11.05	378
20	10.53	1.47	10.81	150
21	10.31	1.25	10.21	376
22	10.81	1.45	10.25	328
23	9.87	1.43	11.64	317
24	9.79	1.36	9.01	313
25	11.15	1.54	10.04	330
26	10.80	1.47	10.18	375
27	10.62	1.42	10.84	316
28	10.85	1.50	11.00	366
29	10.64	1.54	10.54	354
30	9.67	1.43	11.61	331
31	11.20	1.34	9.37	329
32	10.77	1.46	9.66	375
33	10.56	1.48	10.00	328
34	10.62	1.48	11.34	358
35	10.36	1.35	11.40	337
36	11.65	1.38	11.91	355

Table 3a (cont'd)

37	10.53	1.32	10.72	378
38	11.40	1.23	10.37	329
39	11.21	1.48	10.87	371
40	11.89	1.24	10.21	314
41	10.32	1.31	9.35	298
42	10.08	1.11	9.40	321
43	9.46	1.50	10.58	355
44	12.44	1.53	11.49	396
45	10.61	1.49	11.43	315
46	10.56	1.63	10.18	293
47	11.14	1.40	11.83	368
48	10.43	1.45	12.13	371
49	10.93	1.47	11.61	373
50	12.00	1.53	10.63	311
51	10.52	1.24	10.20	371
52	11.40	1.27	9.81	345
53	11.00	1.24	10.16	350
54	9.64	1.47	10.04	396
55	11.50	1.39	10.18	331
56	10.60	1.48	9.75	350
57	10.38	1.39	8.98	311

Table 3.b. Moisture, Ash, and Protein Contents of Whole Ground Flour from Michigan Wheat Varieties from the 2014 Harvest (n=3)

Crop Year 2014				
Variety Number	**Moisture content (%)**	**Ash content (%)**	**Protein content (%)**	**Falling Number (s)**
2	10.38	1.56	11.67	299
5	11.69	1.46	10.20	334
6	11.29	1.54	10.06	370
8	10.01	1.31	10.00	368
9	11.19	1.28	10.60	302
12	11.87	1.57	10.21	332
13	11.36	1.40	10.84	319
14	11.12	1.36	11.12	289
15	10.29	1.59	10.09	297
17	10.12	1.21	10.12	367
21	10.92	1.43	9.81	307
23	9.63	1.21	11.02	325
24	10.29	1.34	11.45	361
27	10.63	1.55	10.37	325
28	10.53	1.30	11.02	272
29	10.63	1.44	9.87	259
30	10.28	1.36	11.72	314
31	9.74	1.50	10.38	345
33	10.27	1.41	11.11	313
34	10.46	1.37	10.11	359
36	9.48	1.37	10.73	262
37	10.75	1.55	11.00	291
38	11.11	1.21	11.38	332
39	11.65	1.24	10.41	307
40	11.43	1.27	10.58	262
41	11.54	1.58	10.87	313
42	10.49	1.38	10.47	278
43	10.10	1.24	10.86	303
45	10.25	1.37	10.55	278
46	10.85	1.35	11.74	269
47	11.76	1.32	11.35	268
49	11.26	1.51	11.43	350
51	10.87	1.42	10.26	347
52	11.47	1.55	10.26	254
58	10.37	1.50	9.55	362
59	10.74	1.29	11.17	326

Table 3b (cont'd)

60	11.96	1.55	10.00	282
61	10.37	1.48	10.37	368
62	10.65	1.31	11.43	344

4.2. Total Phenolic Content (TPC) of Wheat Lines

A preliminary comparison between enzymatic and alkaline digestions was performed (Appendix B), and alkaline digestion was found to be more efficient and convenient, and was used for all phenolic extractions for all types of wheat samples for the rest of the current study.

The total, bound, and free phenolic contents of the 2013 and 2014 wheat varieties harvested for this study were measured by the Folin-Ciocalteu assay using a gallic acid standard for comparison. The colorimetric analysis was used to take into account the cumulative effects of the total combined reducing compounds found in the whole ground flour and the milled fractions, and the specificity of ferulic acid as the main phenolic acid constituent in the wheat samples was measured by RP-HPLC analysis discussed in later sections.

4.2.1. Total Phenolic Content (TPC) of Whole Ground Wheat Flour

The TPC, bound PC and free PC levels of whole ground flour of the 2013 and 2014 varieties from the MSU Wheat Breeding Program selected for this thesis study were examined and expressed as milligrams of gallic acid equivalent (GAE) per gram of whole ground flour (Figures 4 and 5). There were significantly different groups of varieties with noticeable low and high group ranges. The number of significantly different groups decreased from the 2013 to the 2014 harvest for both the bound and free phenolic measurements, alongside an overall increase in TPC in the 2014 harvest, as could be seen in their higher population F value and lower population p value (Appendix A, Table 11). Gallic acid is a popular reference compound

used as the standard for TPC colorimetric assays due to its solubility in water, non-volatility, and stability when exposed to pH and temperature changes.

From the 2013 harvested wheat, varieties with the highest total PC were varieties #34 (F2003) and #52 (Jupiter) at 6.0 and 6.9 mg GAE/g, respectively, and those with the lowest were varieties #17 (F2037) and #6 (F1047) (Figure 4.a) at 3.6 and 3.7 mg GAE/g, respectively. The varieties with the highest bound PCs were #1 (F1014) and #52 (Jupiter) at 4.6 and 5.3 mg GAE/g respectively, while those with the lowest were #9 (F1049) at 1.5 mg GAE/g and #17 (F2037) at 1.4 mg GAE/g (Figure 4.b). For the free PC (i.e., water soluble), the highest values were found for #9 (F1049) and #32 (F2011), while the lowest were found for #18 (F2035), #45 (F2021), and #52 (Jupiter) (Figure 4.c). In general, the samples with relatively higher bound PCs had relatively lower free PCs and vice versa, but such a trend was not observed for all studied varieties. It is also noticeable that the free phenolic range levels were on a much smaller scale than those of the bound fraction. Therefore, the level of bound PC or TPC may be more important for breeding, since the amounts of free phenolic acids in wheat were much smaller.

Among the 2014 varieties, there were 34 lines repeated from the 2013 crops, while 5 lines were new entries. The lowest TPC value was found for variety F2037 (#17) at 4.0 mg GAE/g and the highest was for Unnamed 1 (#59) at 6.0 mg GAE/g (Figure 5.a). The measured bound and free PC levels of the 39 wheat varieties from 2014 also displayed noticeable differences. For bound PC, the greatest measured value was for F2022 (#43) at 4.9 mg GAE/g and the lowest was for Ambassador (#51) at 2.8 mg GAE/g (Figure 5.b). The highest free PC value was from Aubrey (#47) at 1.7mg GAE/g, and the lowest was from F2022 at 0.8 mg GAE/g (#43). There was

no clear correlation (r = -0.1275) between the measured free and bound PC values of the combined years. Similar to 2013 varieties, some 2014 varieties with relatively high bound PC values had relatively low free PC values (varieties #38 and #43), and some of the varieties with relatively low bound PC values had relatively high free PC values (varieties #51 and #58) (Figure 5.c). Although the TPC values were similar between the 2013 and 2014 harvests, 2014 varieties generally had higher bound PC values and lower free PC values than those of the 2013 varieties.

The TPC values obtained in this current study were higher than results from other published studies in which the sample preparation for total ferulic acid content analysis was used for TPC assay (Adom et al., 2003). Adom and colleagues reported bound PC values between 0.8 to 1.5 mg GAE/g, which was significantly lower than those in the current study. Besides the very different wheat genetic traits and growing locations, sample preparation procedure was also not identical. In the current study, samples were extracted with water or 2N NaOH followed by neutralization, and the supernatant was used for TPC assay directly, while in the Adom et al. study, the supernatant of NaOH-digested wheat extracted for HPLC analysis was used for TPC analysis for speed and convenience. Their supernatants contained less total reducing materials available for colorimetric reactions (Adom et al, 2003), presumably a result of some loss of phenolic compounds due to the extraction process. Differences in extraction time may also have led to different colorimetric readings. Adom and colleagues (Adom et al., 2003) utilized a digestion time of 1 hour, but the current experiment could not attain complete digestion of the whole ground flour until 4 hours.

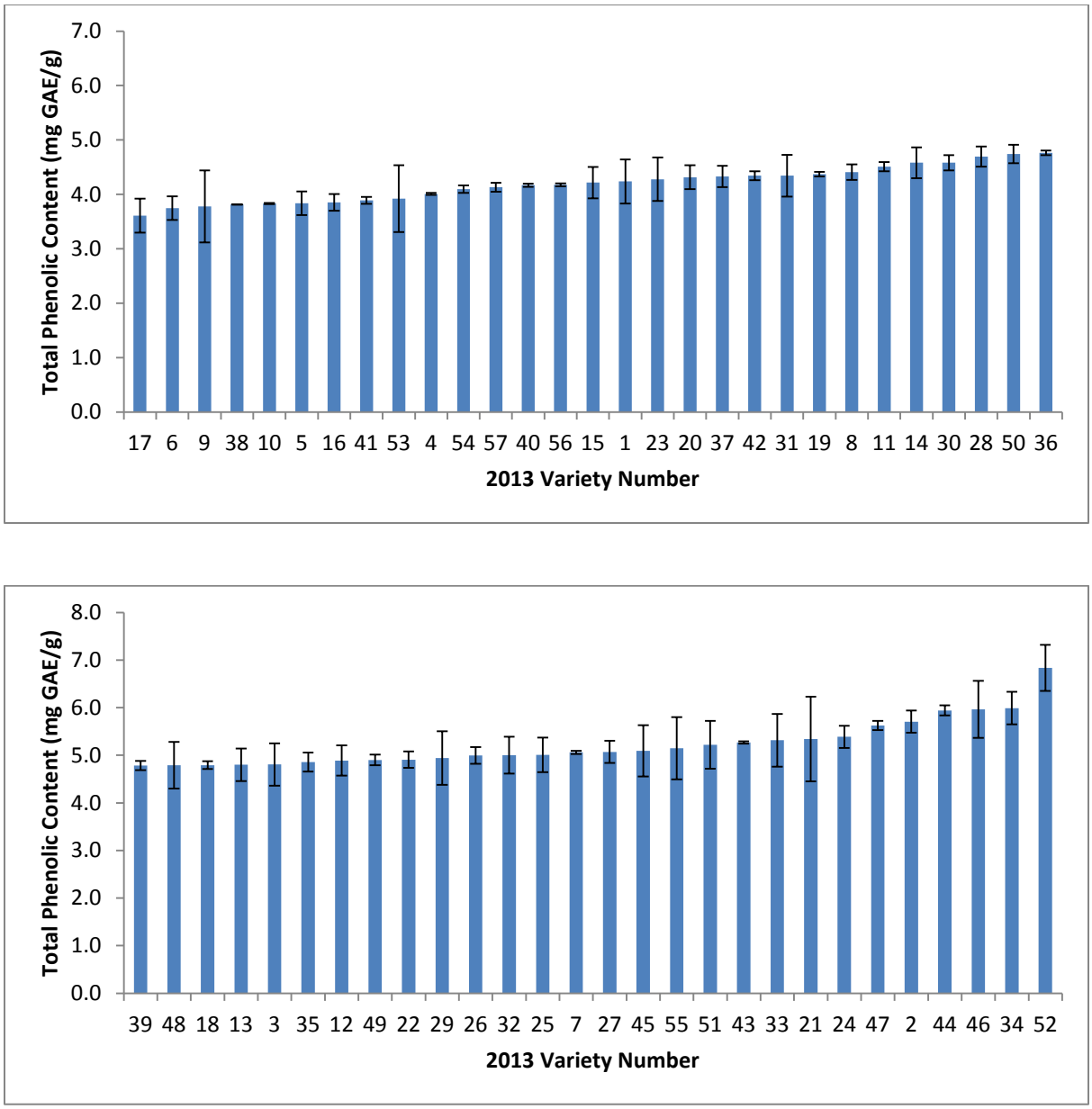

Figure 4.a. Total Phenolic Contents of Wheat Varieties from the 2013 MSU Wheat Breeding Program. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13.

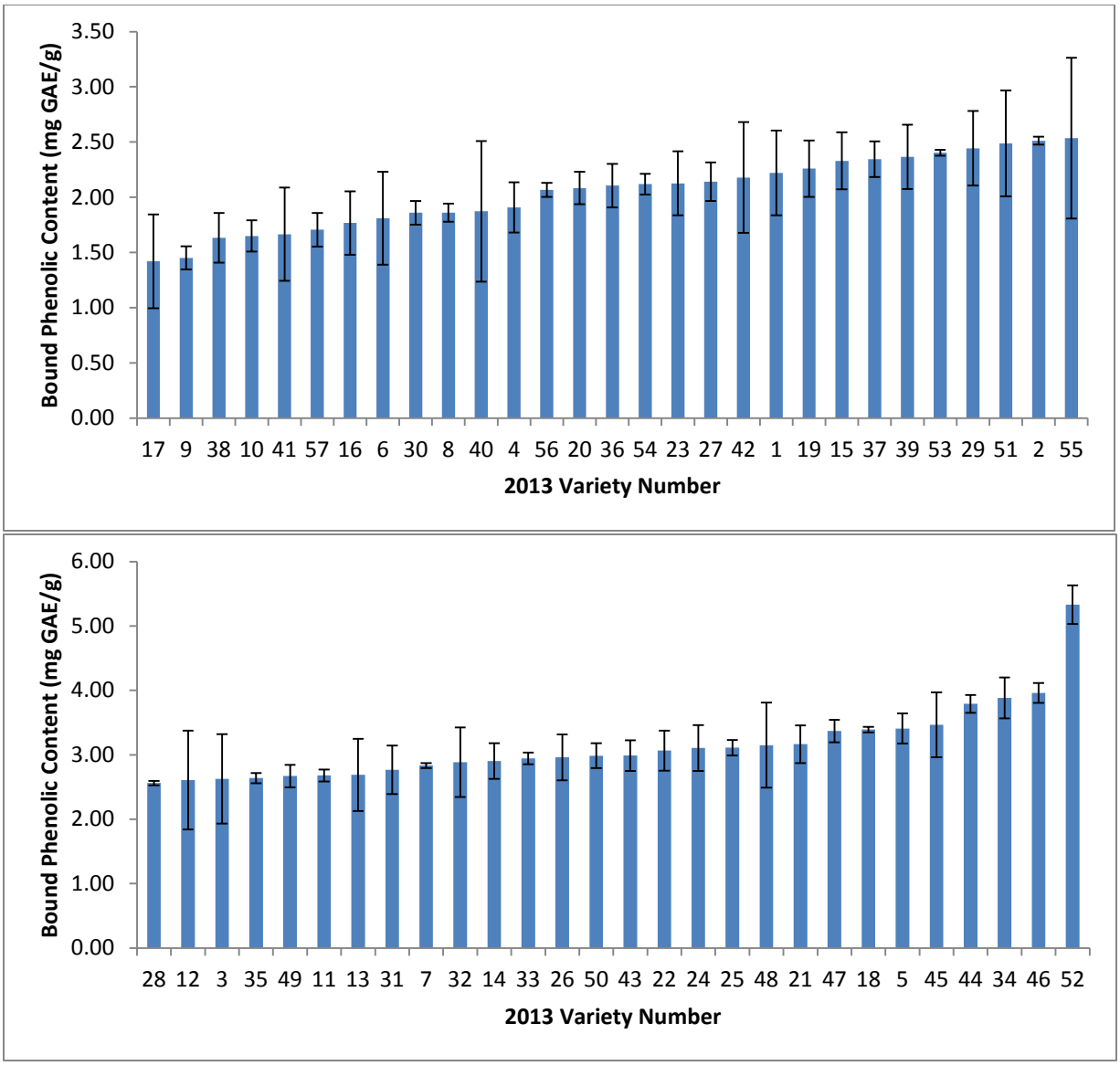

Figure 4.b. Bound Phenolic Contents of Varieties from the 2013 MSU Wheat Breeding Program. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13. Bound phenolic content is obtained by subtracting the free from the total phenolic contents.

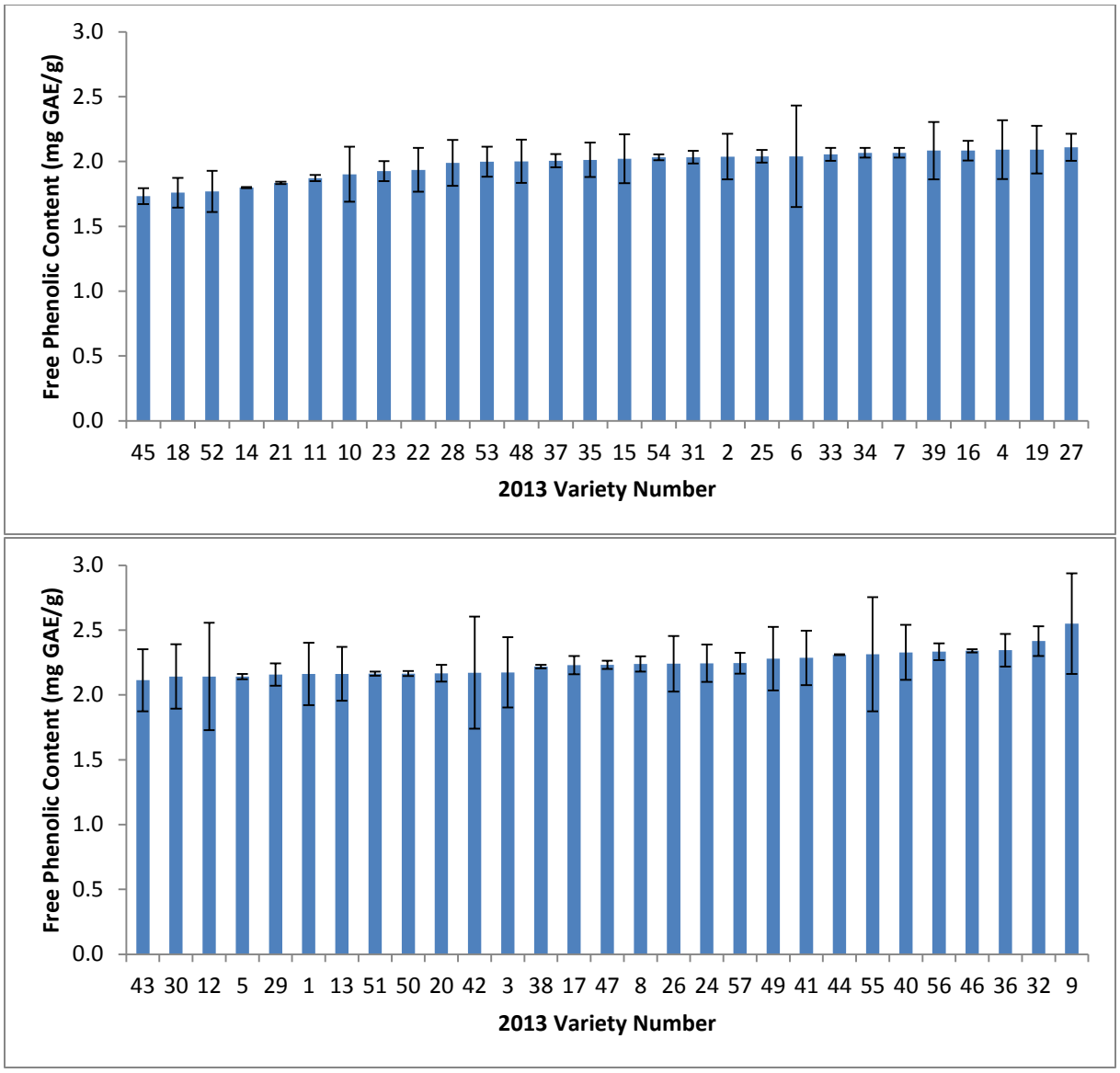

Figure 4.c. Free Phenolic Contents of Lines from the 2013 MSU Wheat Breeding Program. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13.

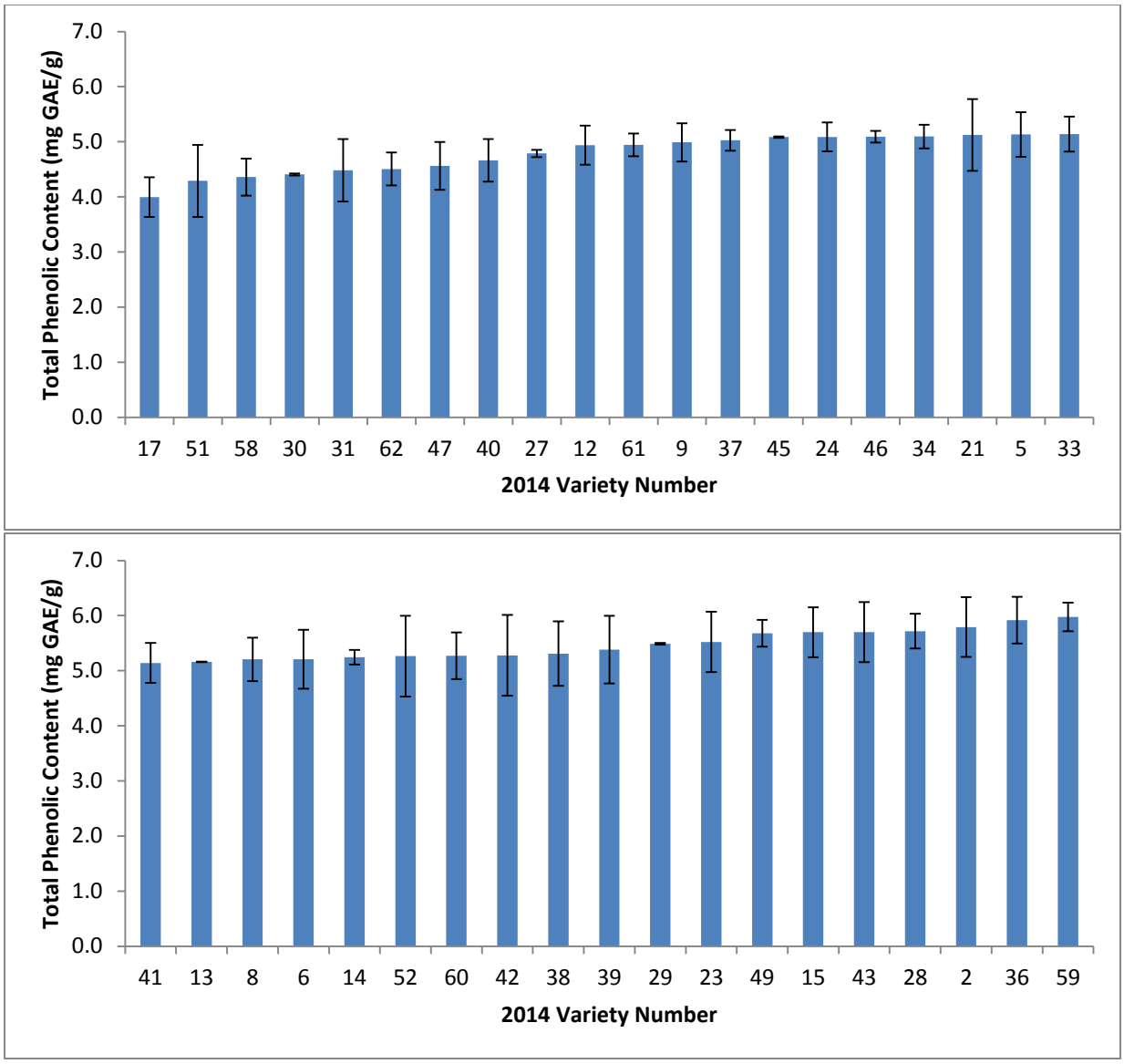

Figure 5.a. Total Phenolic Contents of 2014 MSU Wheat Breeding Varieties. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13.

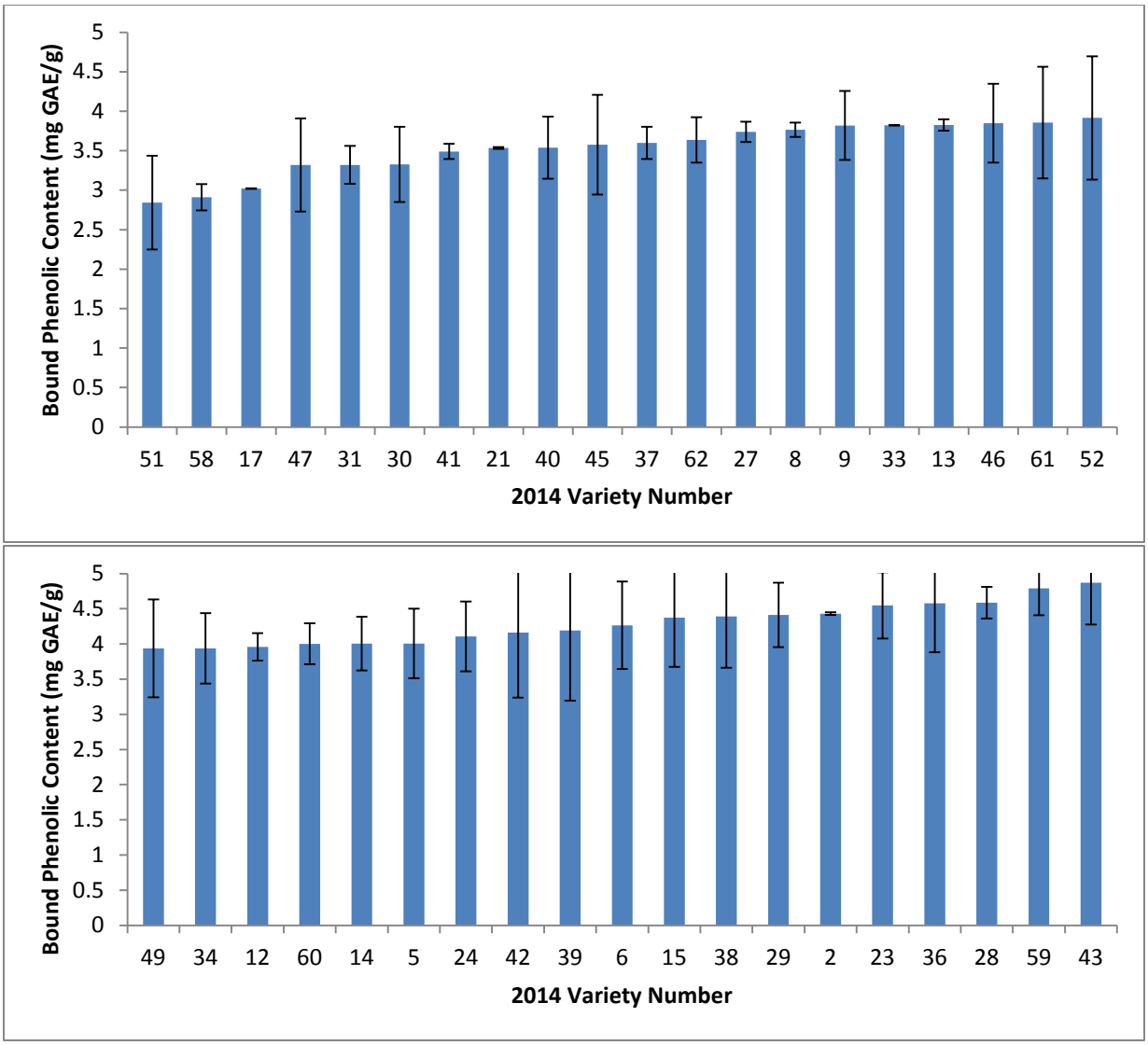

Figure 5.b. Bound Phenolic Contents of 2014 MSU Wheat Breeding varieties. Variety numbers and corresponding names are listed in Table 1. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13. Bound phenolic content is obtained by subtracting the free from the total phenolic contents.

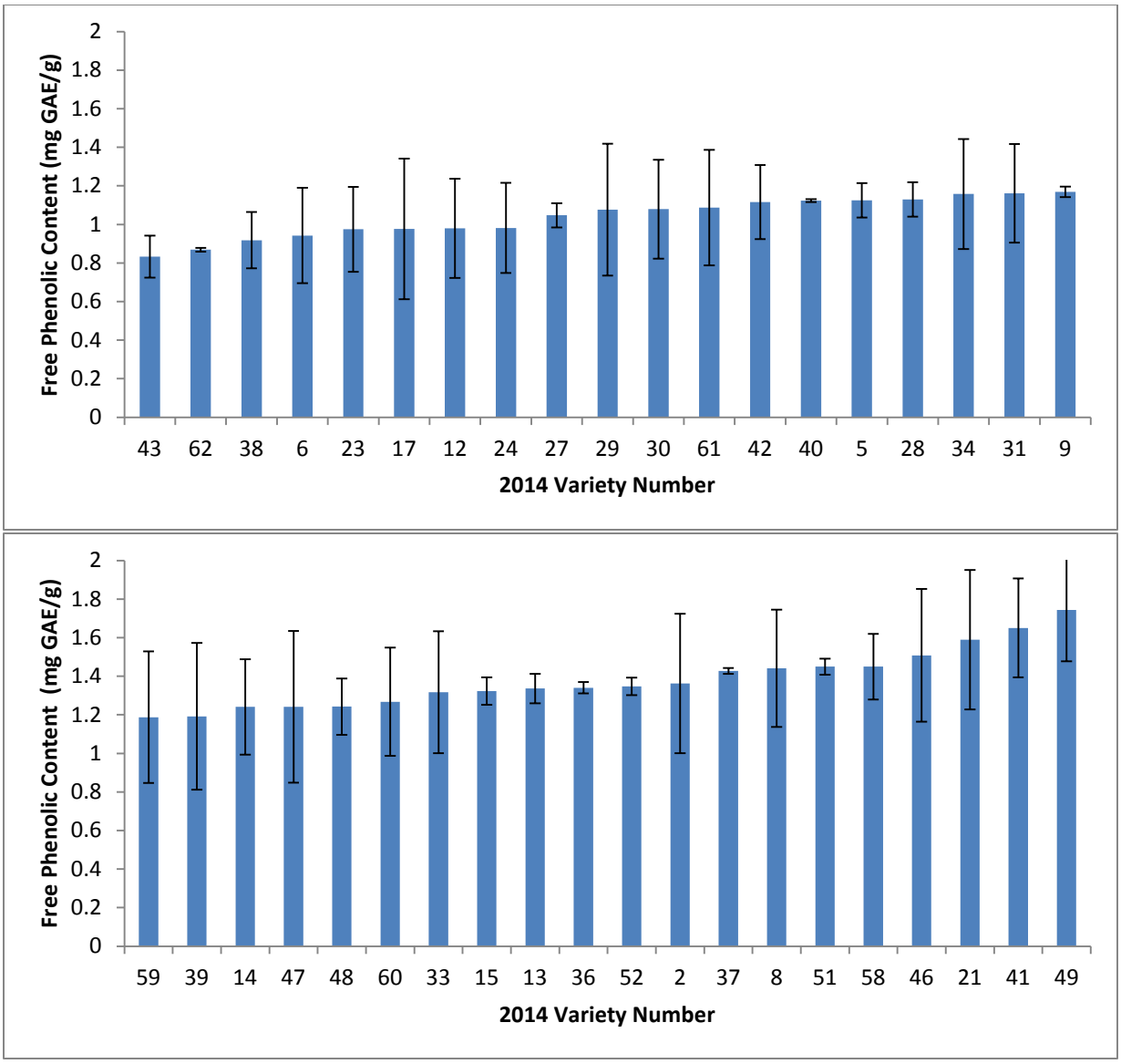

Figure 5.c. Free Phenolic Contents of 2014 MSU Wheat Breeding varieties. Error bars represent one standard deviation of measurements in triplicate. Significant groupings were analyzed with Fisher's Least Significant Difference test ($p<0.05$) and fully presented in Appendix C, Table 13.

4.2.2. Total Phenolic Contents of Milling Fractions of Wheat Varieties

For the TPC analysis of the 2013 and 2014 milled wheat fractions, the bran fractions consistently had the highest phenolic content values, followed closely by the shorts, with flour containing the lowest concentrations. When the TPC values for each studied fraction of the 34 repeated wheat varieties were averaged, the average TPC value of all shorts samples was approximately 80% of the average for all bran, and the average TPC value for all flour samples was approximately 20% of the average for all bran (Figures 6 and 7). For the bran fractions, the highest measured TPC values were for varieties F2040 (#22) and F2011 (#32) from the 2013 harvest (Figure 6), and F2020 (#38) and Jupiter (#52) from the 2014 harvest (Figure 7). The highest measured TPC values from the shorts fraction were for F2034 (#15) and F2011 (#32) from the 2013 harvest, and F1029 (#5) and F2039 (#24) from the 2014 harvest (Figure 7). There were no clear patterns among the TPC values of the three milled fractions (i.e., the TPC levels did not correlate among fractions). The concentration of total phenolic compounds present in one fraction did not seem to affect that present in other fractions. From the results of the wheat harvested in the two years, the ratio of phenolic contents present in the bran, shorts and flour fractions were approximately 5:4:1, respectively.

From these findings, bran and shorts should be considered the ideal starting materials for potential use for commercial phenolic acid extraction utilizing wheat milling byproducts. The above results also indicate that the potential removal of endosperm in future applications would be unlikely to cause any significant loss in phenolic contents due to the relatively lower amount of phenolic acids found in the white flour compared to bran.

It needs to be pointed out that despite the lower concentrations of TP in flour fractions than in shorts and brans, white flour is the major constituent of the wheat milling streams (approximately 70-75%). So even though TP concentration in flour is only about 20% of that in bran, the milled white flour fraction does provide nearly half of the phenolic content of a whole milled wheat sample. The relatively lower concentrations of phenolic acids found in the refined white flour samples suggest that it might not be sufficient to provide meaningful health benefits from phenolic content upon the removal of bran and shorts. This helps explain why whole grain wheat can provide more phenolic compounds, and help promote the physiological benefits of wheat.

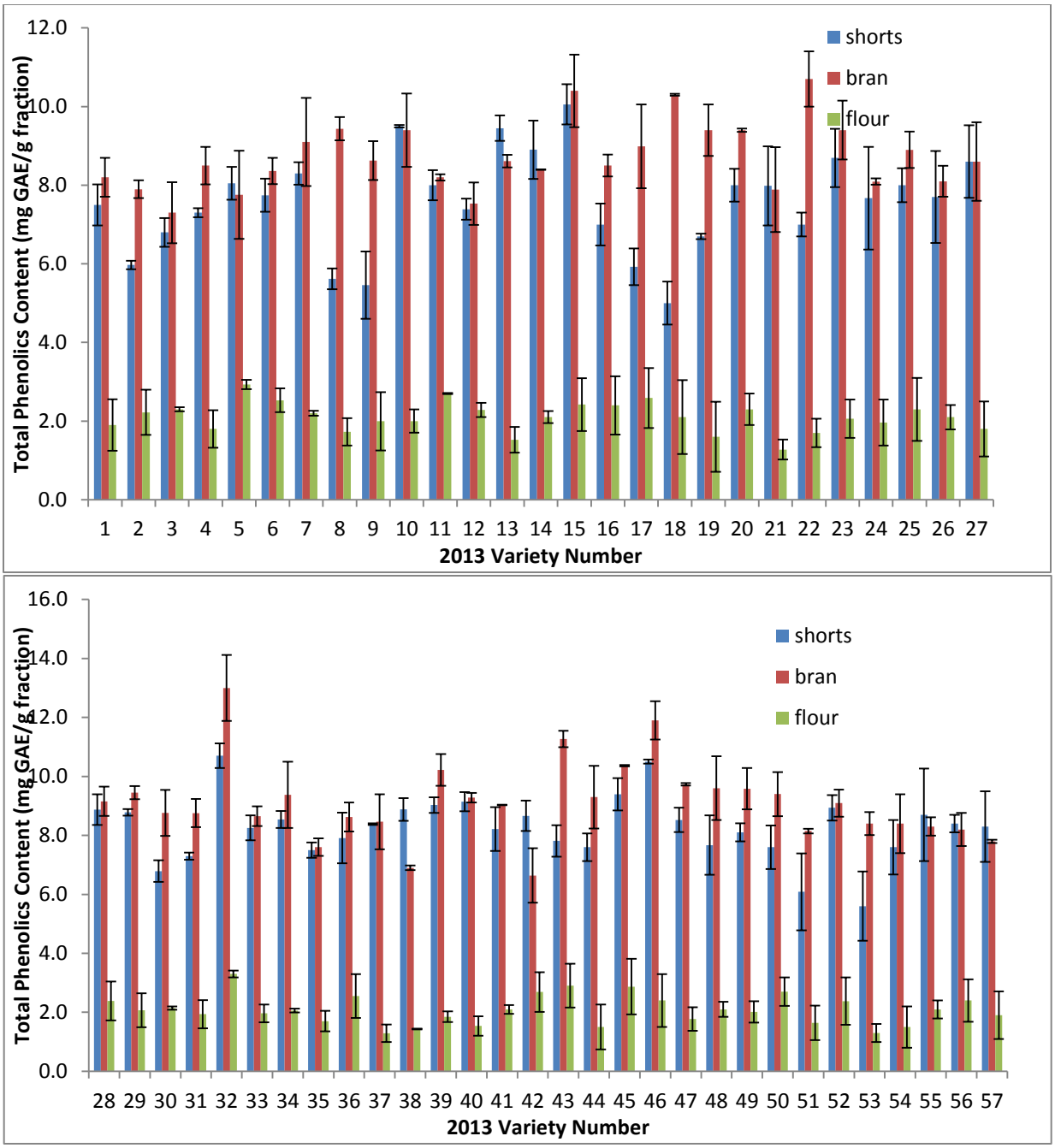

Figure 6. Total Phenolic Contents of Milled Wheat Fractions from Selected Michigan wheat varieties harvested in 2013. Samples were analyzed in triplicate and results are reported as mean values with one standard deviation.

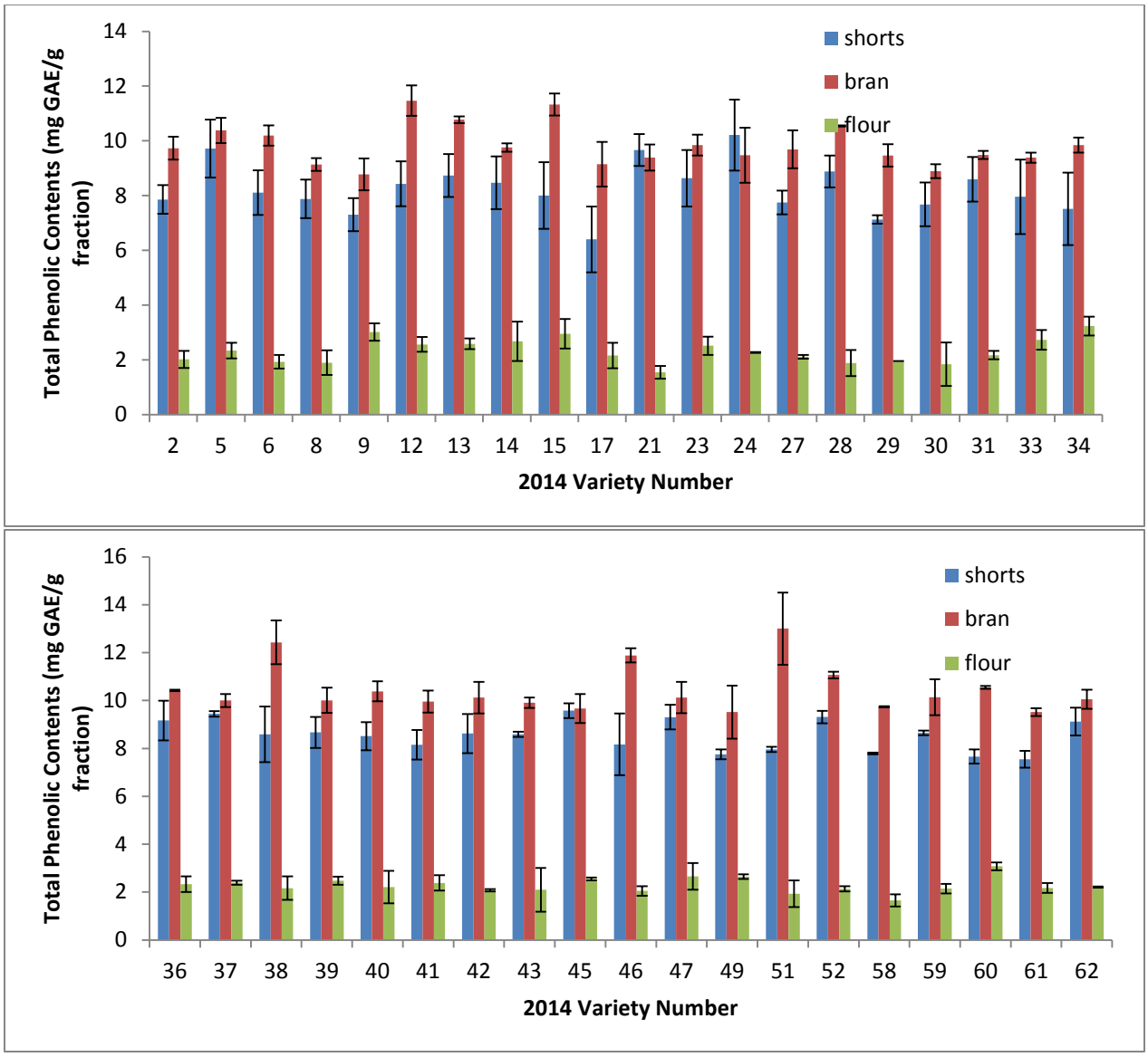

Figure 7. Total Phenolic Contents of Milled Wheat Fractions from Selected Michigan Wheat Varieties harvested in 2014. Samples were analyzed in triplicate and reported as mean values with one standard deviation.

4.3. Ferulic Acid Content of Whole Ground Wheat Flour

Alongside the MSU wheat breeding varieties, three commercial varieties, Aubrey, Ambassador, and Jupiter, were also harvested, milled to whole ground flour, and quantified for ferulic acid contents. For all varieties analyzed, there appeared to be two "populations" of ferulic acids, i.e., bound and free, with bound ferulic acid being the majority. The mean values of the free ferulic acid contents composed only 5% of that of the total ferulic acid for both 2013 and 2014 harvest year varieties (Tables 4 and 5). There did not appear to be any correlation between the amount of bound and free ferulic acids, presumably because the amount of free ferulic acid was too minuscule to indicate any specific pattern. From results in the present study of selected Michigan wheat breeding varieties, the bound (i.e., water insoluble) form of ferulic acid was the only significantly large component of the phenolic compounds found.

Table 4. Ferulic Acid Contents[1] of Selected 2013 MSU Wheat Breeding Varieties

Variety Number	Variety Name	Total FA Content (μg FA/g)	Bound FA Content (μg FA/g)	Free FA Content (μg FA/g)
1	F1014	362 ±6	351 ±11	11.4 ±5.4
2	F1026R	302 ±6	290 ±8	12.5 ±1.8
3	F1012	310 ±24	297 ±17	13.5 ±7.1
4	F1003R	250 ±9	234 ±6	16.4 ±3.7
5	F1029	326 ±7	314 ±9	12.4 ±2.3
6	F1047	288 ±16	274 ±11	14.4 ±5.6
7	F1032R	309 ±3	298 ±2	11.4 ±1.1
8	F1027	350 ±2	336 ±2	13.6 ±0.4
9	F1049	309 ±9	298 ±12	11.0 ±2.9
10	F1051	261 ±1	240 ±1	21.5 ±0.4
11	F1050	358 ±13	328 ±17	30.1 ±3.7
12	F1048	337 ±27	314 ±35	23.5 ±7.9
13	F2031	313 ±3	299 ±4	13.6 ±1.2
14	F2033	272 ±3	246 ±2	25.7 ±1.2
15	F2034	293 ±1	282 ±1	11.5 ±0.4
16	F2032	373 ±22	357 ±2	15.6 ±6.0
17	F2037	314 ±2	294 ±1	19.6 ±0.6
18	F2035	360 ±33	348 ±29	12.5 ±11.9
19	F2041	341 ±13	320 ±9	21.5 ±3.8
20	F2036	253 ±11	240 ±16	12.6 ±4.5
21	F2038	297 ±2	285 ±1	11.6 ±0.7
22	F2040	257 ±12	238 ±17	19.5 ±4.5
23	F2042	371 ±14	345 ±18	25.6 ±4.1
24	F2039	412 ±1	399 ±1	12.7 ±0.4
25	F2001R	326 ±30	308 ±20	18.5 ±10.0
26	F2006	235 ±12	216 ±17	19.4 ±5.2
27	F2005	318 ±3	293 ±4	25.2 ±1.0
28	F2015	370 ±12	355 ±16	15.4 ±3.2
29	F2009	321 ±7	304 ±5	17.5 ±2.4
30	F2012	285 ±30	272 ±44	12.9 ±14.3
31	F2008	339 ±4	312 ±5	27.5 ±1.3
32	F2011	365 ±26	348 ±13	17.4 ±7.0
33	F2014R	283 ±12	267 ±8	16.4 ±4.2
34	F2003	390 ±5	374 ±4	15.8 ±1.3
35	F2004	355 ±19	332 ±24	23.5 ±5.3
36	F2002	318 ±8	302 ±10	15.9 ±2.5
37	F2028R	325 ±10	306 ±13	18.7 ±3.1
38	F2020	293 ±22	285 ±14	8.5 ±7.6
39	F2024R	265 ±1	249 ±1	15.6 ±0.5
40	F2018	365 ±10	339 ±13	26.4 ±2.7
41	F2019	285 ±15	274 ±19	11.5 ±5.2
42	F2016	289 ±26	274 ±15	14.6 ±8.9
43	F2022	397 ±11	383 ±14	13.7 ±2.6
44	F2025R	290 ±1	272 ±1	18.5 ±0.2

Table 4 (cont'd)

45	**F2021**	**310**	**±40**	**290**	**±34**	**20.3**	**±12.9**
46	F2030	303	±22	282	±35	21.5	±7.1
47	F2029R	343	±21	316	±15	26.8	±6.1
48	F2027R	339	±3	320	±2	19.5	±1.0
49	Aubrey	312	±12	297	±16	14.7	±4.0
50	Hopewell	357	±25	340	±33	17.3	±6.9
51	Ambassador	357	±12	338	±16	19.4	±3.8
52	Jupiter	337	±34	322	±24	14.7	±10.1
53	Red Ruby	329	±15	304	±21	25.3	±4.6
54	VA09W-188WS	272	±12	257	±17	14.6	±4.3
55	Cayuga	334	±13	317	±17	17.4	±3.9
56	VA09W-192WS	265	±19	251	±12	13.7	±7.0
57	Caledonia	325	±9	312	±12	12.6	±2.8

[1] Results are expressed as micrograms of ferulic acid standard equivalent per gram of whole ground flour, with the data presented as means ± standard deviation (n=2). For statistically significant groupings of data see Appendix C, Table 13.

Table 5. Ferulic Acid Contents[1] of Select 2014 MSU Wheat Breeding Varieties

Variety Number	Variety Name	Total FA Content (μg FA/g)	Bound FA Content (μg FA/g)	Free FA Content (μg FA/g)
2	F1026R	388 ±4	370 ±7	18.2 ±3.4
5	F1029	419 ±49	399 ±49	19.3 ±0.8
6	F1047	387 ±39	351 ±61	36.0 ±7.8
8	F1027	375 ±13	353 ±1	22.4 ±11.4
9	F1049	373 ±2	356 ±1	17.6 ±0.8
12	F1048	403 ±10	388 ±10	15.1 ±0.2
13	F2031	385 ±20	363 ±23	21.9 ±3.1
14	F2033	338 ±11	307 ±7	31.4 ±4.0
15	F2034	397 ±20	373 ±29	24.0 ±8.6
17	F2037	370 ±3	353 ±3	17.3 ±0.1
21	F2038	413 ±9	399 ±14	14.3 ±5.2
23	F2042	346 ±15	325 ±11	21.1 ±4.0
24	F2039	457 ±41	429 ±22	28.0 ±18.7
27	F2005	318 ±1	304 ±0	14.1 ±1.2
28	F2015	356 ±7	341 ±6	15.4 ±1.2
29	F2009	403 ±19	387 ±19	16.1 ±0.5
30	F2012	429 ±0	415 ±1	14.6 ±0.7
31	F2008	390 ±15	372 ±16	18.3 ±1.6
33	F2014R	457 ±24	435 ±34	22.9 ±0.5
34	F2003	432 ±35	415 ±45	17.5 ±0.3
36	F2002	368 ±20	343 ±25	25.1 ±15.5
37	F2028R	467 ±34	442 ±41	25.0 ±7.3
38	F2020	373 ±2	356 ±3	16.4 ±4.8
39	F2024R	382 ±27	363 ±26	18.2 ±1.3
40	F2018	368 ±36	344 ±39	23.2 ±3.5
41	F2019	353 ±30	329 ±31	24.2 ±1.1
42	F2016	396 ±10	375 ±9	20.7 ±0.4
43	F2022	360 ±51	341 ±43	19.4 ±2.2
45	F2021	381 ±21	349 ±27	32.9 ±6.2
46	F2030	421 ±28	390 ±43	31.2 ±15.0
47	F2029R	452 ±26	429 ±29	22.5 ±2.5
49	Aubrey	334 ±6	303 ±23	30.9 ±17.0
51	Ambassador	324 ±1	305 ±3	18.8 ±2.0
52	Jupiter	316 ±36	298 ±34	18.6 ±2.3
58	MSU Line F2010	371 ±9	351 ±21	20.0 ±12.0
59	Unnamed 1	391 ±36	374 ±32	16.9 ±3.2
60	Unnamed 2	366 ±1	347 ±6	18.7 ±5.6
61	Unnamed 3	422 ±42	407 ±44	15.9 ±2.2
62	F0013R	368 ±38	330 ±33	37.8 ±4.7

[1]Results are expressed as micrograms of ferulic acid standard equivalent per gram of whole ground flour, with the data presented as means ± standard deviation (n=2). For statistically significant groupings of data see Appendix C, Table 13.

The range of the total ferulic acid contents was between 235 μg FA/g and 412 μg FA/g for 2013 varieties (Table 4), with a 57% difference between the maximum and minimum measured values. The range of total ferulic acid contents was between 316 μg FA/g and 467 μg FA/g for 2014 varieties (Table 5), with a 68% difference between the most extreme values. Among the 2013 wheat varieties, the highest values were for varieties F2039 (#24) and F2022 (#43), while the lowest values were for F2036 (#20) and F2006 (#26) (Table 4). For the 2014 wheats, varieties with the highest measured total ferulic acid contents were F2039 (#24), F2028R (#37), and F2029R (#47), while those with the lowest were F2005 (#27), Ambassador (# 51), and Jupiter (#52) (Table 5). These ferulic acid content ranges were found to be consistent with the findings reported by Adom and colleagues (Adom et al., 2003), and fall within their measured range of between 250 μg/g and 513 μg/g for durum and soft wheat varieties harvested from the Eastern United States. Mpofu and colleagues reported similar results for hard wheat varieties from Western Canada, at between 371 μg/g and 441 μg/g (Mpofu et al., 2006). Moore and colleagues (2005) measured soft wheat varieties grown in Maryland, USA, and their total ferulic acid contents ranged between 455.5 μg/g and 621 μg/g, which was a higher range than many of the values measured in the current study, even though all of the studied Michigan varieties were soft wheats as well.

The commercial varieties were all among the wheat varieties with lower measured ferulic acid contents for both crop years (Tables 4 and 5). In the 2013 harvest, Aubrey had a total ferulic acid content of 312 μg/g of whole ground flour, Ambassador had a total ferulic acid content of 358 μg/g, and Jupiter a content of 338 μg/g. In the 2014 harvest, Aubrey had a total ferulic acid content of 334 μg/g, Ambassador had a total ferulic acid content of 324 μg/g, and

Jupiter one of 316 μg/g. On average, Aubrey had a slight increase in ferulic acid content in 2014, while Ambassador and Jupiter showed slight decreases. In other words, a vast majority of the breeding varieties studied showed higher ferulic acid contents than some commercially grown soft winter wheat varieties in Michigan. This information may be helpful for breeders to identify potential early lines with higher ferulic acid and total phenolic contents in their breeding programs.

As expected, the ferulic acid (FA) contents of the varieties were different from each other in their measured values. The total phenolic contents were higher than the total ferulic acid contents, due to minor contributions from other phenolic compounds. The FA levels had their highest and lowest values with different varieties. The commercial variety Ambassador (variety # 51) was among the lower FA values, while the values for Aubrey and Jupiter (varieties #49 and #52) were close to the median value (Tables 4 and 5). Out of the three commercial varieties, Ambassador was consistent in having among the lowest values for both ferulic acid content and total phenolic content out of the 2014 studied varieties.

4.4. Pearson Correlations of the Phenolic and Ferulic Acid Contents of Studied Varieties

For further examination of the results of TPC by colorimetric analysis, the Pearson product-moment correlation coefficient was calculated between total and bound phenolic contents of the combined two harvest seasons using Microsoft Excel; this coefficient was 0.97 (n=34), indicating a strong positive relationship. This confirms that the TPC was mainly impacted by the bound PC. On the other hand, the correlation coefficient between the total phenolic contents and the respective free phenolic contents for the whole ground flour of the studied varieties was -0.1, which demonstrated no correlation. These results show that the amount of free phenolic compounds could not indicate the amount of bound phenolic compounds present for a given variety. In fact, whether a variety contained relatively high or low amounts of bound phenolic acids, the free phenolic content remained negligible, ranging between about 5 and 10%. If the bound phenolic acids in the flour samples were actually being released into free form through enzymatic or chemical hydrolysis over time, a pattern of ratios between the bound and free phenolic contents could not be found in this study to support this hypothesis. The free phenolic contents of most varieties studied were close to 2.0 mg GAE/g, whereas the values of the bound phenolic contents were much more varied and had larger ranges.

For the whole ground wheat flour samples, the Pearson product-moment correlation coefficient between the total phenolic contents measured by the Folin-Ciocalteu assay and the total ferulic acid contents measured by RP-HPLC was 0.26 (n=35), which indicated a weak positive relation between the two sets of data. Most varieties with relatively high total phenolic content were also relatively high in ferulic acid content, but the varieties with the highest or

lowest recorded values were not the same for the two methods studied. The main reason for the discrepancies in results between the two methods could be the specificity for and range of the compounds that were measured and identified. The Folin-Ciocalteu assay measures the entire reducing capacity of the sample, mainly phenolic compounds in plant materials such as wheat. That means all phenolic compounds will contribute to the results, and any kind of non-phenolic compounds may react and influence the readings. Such compounds could be other types of antioxidants present in wheat, such as carotenoids and, proteins, thiols and free sugars, and tocopherols (Moore et al., 2005). For some of the wheat varieties examined in the current study, clear differences in flour suspension color brightness were noticed during alkaline hydrolysis of bound phenolic contents, which suggested that the varieties could have contained different types of non-phenolic antioxidants which reacted differently when exposed to the alkaline pH environment. This discrepancy between actual measured substances may help explain the poor correlation between total ferulic acid and total phenolic content among the wheat varieties studied.

Overall, the studied Michigan wheat varieties had phenolic acid contents similar to those measured and reported elsewhere in the United States (Adom et al., 2003). High and low phenolic acid ranges were found, although, the differences were not extreme. However, that did not dismiss the possibility of the existence of genetic far outliers that may still exist within the Michigan wheat breeding varieties.

4.5. Effect of Crop Year on Ferulic Acid Content of Whole Ground Wheat Flour Samples

Of the studied wheat varieties, a total of 34 were planted and harvested in both 2013 and 2014 in the central Michigan region of Saginaw and Ingham. They were compared for annual changes in ferulic acid contents. Out of 34 comparable varieties, 29 varieties showed increased total ferulic acid (FA) content in the 2014 harvest when compared to the 2013 harvest, with 14 varieties having increases greater than 30%, and 4 varieties showing small decreases of up to 10%. The average total FA content of the 34 comparable varieties harvested in the two crop years showed an increase of 23% from 2013 to 2014, from 314 μg FA/g whole ground flour to 386 μg FA/g, respectively. From one-way ANOVA analysis, the measured total FA values of the 2013 and 2014 crops studied were statistically significantly different ($p<0.001$). It is well known that plant traits are generally governed by genetic and environmental factors, and it is not surprising that the crop year, as an external factor, asserted significant impact on the phenolic contents (i.e., FA in the current study) of the wheat kernels. Since these wheat varieties were planted in the same experimental location with controlled fertilizer applications, the weather conditions became the most important variations for consideration.

Soil and weather conditions for the planting sites of the east-central region of Michigan were acquired from the Michigan State University Enviro-weather Automated Weather Station Network (MAWN; http://www.agweather.geo.msu.edu.proxy1.cl.msu.edu/mawn/mawn.html), between the months of September from the previous year, at planting, to June of the harvest year (select data listed in Table 6; for all data, please see the website data listed for east-central Michigan from September 1, 2012 through June 31, 2013, and from September 1, 2013 through

June 31, 2014). In the crop year of 2013, the highest recorded daily air temperature for 24 hours was 14.7^{o}C to 33.1^{o}C, while the lowest recorded single day air temperature range was -11.7 ^{o}C to -7.8^{o}C. For the growing seasons of crop year 2014, the highest recorded daily air temperature range was 10.9^{o}C to 30.9^{o}C, while the lowest recorded 24-hour range was -25.3^{o}C to -17.0^{o}C. From these data, the temperature range showed a much colder winter in 2014. The measured cumulative rainfall was 534 mm in the 2013 crop growing period, and dropped by 55% to 242 mm for the 2014 crop growing period. In the 2013 planting season, the highest measured daily soil moisture at 10.16 cm depth was 0.947-1.082 cm water/cm soil, and the lowest daily soil moisture was 0.274-0.277 cm water/cm soil. In the 2014 planting, the highest soil moisture measured at 10.16 cm depth was 0.980-0.986 cm water/cm soil, while the lowest soil moisture was 0.345-0.345 cm water/cm soil. The average soil moisture had increased from 0.546 cm water/cm soil in 2013 to 0.622 cm water/cm soil in 2014.

There are many environmental factors that can cause changes in ferulic acid content in many plant species, such as high irradiation, heat or chilling, excessive change in moisture caused by droughts or flooding, and nutrient deficiency (Schützendübe and Polle, 2002). For the soil moisture, a decrease in soil moisture could decrease the nutrient absorption of crops (Metwally and Pollard, 1959), and the subsequent mineral deficiency could signal increased production of phenolic acids in laboratory situations (Marschner, 1991). Both the lowest measured soil moisture and the average soil moisture had increased from 2013 to 2014. Thus, soil moisture was probably not a main factor behind the changes in the total ferulic acid of the studied wheat varieties of two crop years. Decreased rainfall could lower the overall water content in the crops and cause drought- and saline-induced stress responses, but this can be

compensated for by increased watering and/or irrigation. The lack of decrease in soil moisture showed that the decrease in rainfall did not seem to be a factor. Moreover, Mpofu and colleagues found no correlation between the average rainfall and the phenolic contents of different wheat varieties grown in Western Canada (Mpofu et al., 2006).

Members of the grass family increase the production of phenolic compounds to counter cold-induced oxidative stress (Sarkar et al., 2009). Decreases in air temperature have also been known to increase the presence of phenolic compounds in non-cereal plants (Rivero et al., 2001). Michigan had experienced an unusually cold winter in the winter prior to harvest season of 2014. Since the other soil and weather factors have been eliminated as major contributors, it is possible that the low average daily air temperatures experienced for the 2014 crop from October 2013 to March 2014 had a direct effect on the ferulic acid content of the studied Michigan wheat varieties. This indicates that crops adapted to colder climates, such as winter wheat, may possess superior genetic capacity for increased phenolic acid biosynthesis compared to crops grown in warmer climates, as part of their adaptation strategy.

Table 6. Select Environmental conditions of Growing Locations in East-Central Michigan from the Harvesting and Planting Seasons of 2013 to 2014 (MAWN, 2012, 2013, 2014)

Year of Harvest	Maximum measured single day average air temperature (^{o}C)	Minimum measured single day average air temperature (^{o}C)	Total Rainfall (cm)	Minimum Measured Soil moisture (cm water/cm soil)	Average Daily Soil Moisture (cm water/cm soil)	Average Crop Total Ferulic Acid content (μg FA/g)[1]
2013	33.1	-9.75	53.42	0.277	0.546	290
2014	30.9	-21.15	24.23	0.345	0.622	316

[1]Average of 34 wheat varieties studied; μg FA/g whole ground flour.

4.6. Baking Quality of Cookies after Fortification with Free and Bound Forms of Ferulic Acid

A model system of white flour for cookie baking was used to help understand the stability of free and bound forms of ferulic acid during simple processing like cookie baking. Ambassador was used as the representative variety for the cookie baking trials. All cookie treatments were baked in triplicate. The cookie moisture content, cookie color, dimensions, and hardness were examined after baking to compare the baking qualities of the different flour blends with and without added ferulic acid.

4.6.1 Visual Quality of Cookies Baked from Flour Fortified with Free and Bound Forms of Ferulic Acid

Cookies made from the treatment flour blends with the 20% bran and the free FA powder most resembled the control white flour cookie visually by possessing similar distribution of brown spots and surface ridging (Figures 8.b and 8.d, respectively). Cookies from the treatment using the 4.4% arabinoxylan blend were noticeably smoother in texture (Figure 8.c). The treatment with the free FA powder produced cookies with slight edge darkening (Figure 8.d), while cookies made from the flour blend with the digested 20% bran and free FA powder showed significant edge darkening (Figure 8.e), with noticeable dark brown and black spots spread throughout its surface.

Figure 8. Representative Visual Appearance of Cookies baked using Different Flour Blends Consisting of white flour and: (a) No additions, (b) 20% bran, (c) 4.4% arabinoxylan, (d) Free FA powder, and (e) Digested 20% bran with free FA powder.

4.6.2. Effect of Ferulic Acid Source on Cookie Moisture Contents

Ambassador flour was used as the representative variety. The highest cookie moisture was observed for cookies baked from the flour blend with 4.4% wheat arabinoxylan as a source of ferulic acid fortification (Table 7). The moistures of the baked cookies made from the 4.4% arabinoxylan flour blend and the 20% bran flour blend were significantly higher than the moisture contents of the baked cookies from the control and the other flour blends. In addition, there were no significant differences in baked cookie moisture among cookies made from the control flour and the flour blends with free FA or with 20% digested bran and free FA (Table 7). There was strong positive Pearson correlation of 0.98 between the combined final cookie moisture content and the cookie height (Appendix A, Table 11). It is believed that the fortification with ferulic acid by using "native" bran (which contains arabinoxylan, i.e., the 20% bran flour blend) or insoluble arabinoxylan isolated from wheat bran (i.e., the 4.4% arabinoxylan flour blend) resulted in markedly higher cookie moistures because of the high water absorption capability of arabinoxylan. Presence of the bran that had been treated with NaOH hydrolysis to remove bound ferulic acid then supplemented with FFA (i.e., the 20% digested bran plus free FA flour blend) did not seem to have a large impact on water absorption and final moisture content of the cookies. It is possible that the alkaline treatment and heating had decreased the water absorption capability of arabinoxylan.

Table 7. Moisture Contents[1] of Cookies Baked From Different Ambassador Wheat Flour Blends

Sample Number	Flour Blend	Cookie Moisture (%)
1	White flour (control)	3.53 ± .028a
2	20% Bran	6.54 ± 0.021b
3	4.4% Arabinoxylan	6.83 ± 0.106b
4	Free ferulic acid	3.37 ± 0.085a
5	20% Digested bran plus Free ferulic acid	3.25 ± 0.018a

[1] Values with the same letter are not significantly different from each other (α=0.05, n=3).

6.3. Effect of Ferulic Acid Source on Cookie Color

Color analysis was performed on the different flour blends from the Ambassador flour. The color and lightness of cookie samples were measured using the LAB Colorspace scale, corresponding to the value L, where 0 is the darkest and 100 is the lightest. The opposing color axis was measured using a* and b*, which showed the cookies as all within the brown color region. The lightest baked cookies were from the flour blend fortified with wheat arabinoxylan powder at 73.2 lightness (L) units, and from the 20% bran flour blend at 70.4 lightness units (Table 8). The cookies with the lowest LAB values were from the flour blend with 20% digested bran flour fortified with free FA powder, which was significantly different from all other samples. The LAB values of cookies baked from each of the treatment flours were also significantly different from that of cookies baked from the control white flour. The cookies with the lightness values closest to that of the control cookie were those made from the 20% bran flour blend, which was 3 units lighter, and from the white flour fortified with FA powder, which was 3 units darker. For the cookie color axis (a* and b*), all of the flour blend cookies, except those made from the free FA blend, were significantly different from the control in color.

In cookie baking, the main factor in changes to cookie color is due to non-enzymatic browning. This process is moisture sensitive, as the increased presence of even small amounts of water in foods during cooking can drastically reduce the degree of Maillard reaction (Peterson et al., 1994). This could explain the significantly lighter color of the bran cookies and the arabinoxylan cookies in the present study, as both of these cookie samples also had significantly higher moisture contents (Table 7).

Table 8. Color Analysis[1] of Ferulic Acid-Fortified Cookies made from Ambassador flour

Sample Number	Flour Blend	LAB Colorspace Units		
		L*	a*	b*
1	White flour (control)	67.44 ± 2.46b	10.91 ± 1.23b	38.02 ± 0.55a
2	20% Bran	70.38 ± 1.02a	9.92 ± 0.49c	37.09 ± 1.06b
3	4.4% Arabinoxylan	73.21 ± 1.43a	7.94 ± 0.81d	35.80 ± 0.49c
4	Free ferulic acid	64.56 ± 2.90c	11.05 ± 1.79b	37.62 ± 1.50a
5	20% Digested bran + free ferulic acid	53.72 ± 0.74d	11.915 ± 0.34a	32.76 ± 0.58d

[1]Values not sharing the same letter in the same column are significantly different from each other ($p<0.05$). L* indicates lightness, a* represents the red/green color axis, and b* represents the yellow/blue axis.

Non-starch polysaccharides have been known to pull water away from the flour (James et al., 1989), but the bran and arabinoxylan cookies did not show increased browning even though white flour was still the majority ingredient for all the flour blends. Therefore, the amount of water held by the non-digested bran and arabinoxylan may have been either redistributed to the white flour later on during baking, or the quantity of water added in the cookie formulation was already above the absorption limit of the white flour portion, with the unabsorbed water taken up into the bran and arabinoxylan polysaccharides.

The browning of the digested bran blend cookies was very extreme, being much higher than the slight browning of the FA powder blend cookies, despite being similar in moisture content relative to the cookies baked from the FA flour blend. This indicates that one or more enzymatic reactions had possibly taken place. Polyphenol oxidase is a common browning enzyme in plants and is present in many wheat varieties (Demeke and Morris, 2002). In the literature, enzymatic browning has been reported by others in wheat-based food products such as noodles (Fuerst et al., 2006). From studies performed by Yang and colleagues, polyphenol oxidase was stable at pH 5-11, and temperatures of up to 70 ^{o}C (Yang et al., 2000). The temperature and pH conditions of the bran alkaline digestion and drying process in Section 3.2.7 were well within the ranges to permit retention of polyphenol oxidase in the digested bran, allowing for a small time window for enzymatic browning. The non-digested bran must have contained identical amounts of the same browning enzymes, but they were not able to react with the bound ferulic acid to produce browning. It is possible that the undigested bran cell wall may have remained structurally stable for sufficient time to prevent the release of intracellular enzymes until their heat inactivation, preventing their reaction with other cookie

ingredients, thereby limiting the amount of browning that occurred. Ferulic acid begins to decompose at 203^{o}C (Fiddler et al., 1967), and the cookie baking temperature of this experiment was 204^{o}C. The breakdown of ferulic acid may have released its phenolic components, which facilitated reaction with PPO.

It was also observed in the present study that the color lightness (L*) and redness (a*) appeared to be related to the form of ferulic acid, free vs. bound. Compared to the values for the control sample of white flour cookies, the addition of free FA (samples #4 and #5) increased both darkness and redness values, while the cookies with added bound FA at the same levels (samples #2 and #3) had reduced darkness and redness. Besides the impact of water holding capacity of arabinoxylan (James et al., 1989) and higher cookie moisture discussed above, the form of FA may play a role in affecting the Maillard reaction, with bound FA being less available to react with the other flour ingredients, as evidenced by the cookies baked with the non-digested bran flour blend.

Using the Pearson correlation equation, there was a weak positive correlation of 0.38 between the decrease in total cookie ferulic acid content and decrease in cookie color lightness (Appendix A, Table 11).

4.6.4. Cookie Diameter and Height

From the cookie dimension measurements, cookies baked from flour blends with the arabinoxylan and with the bran had statistically significantly greater cookie heights at 1.3 cm

and 1.2 cm, respectively, than the other three cookie samples, which had similar lower heights that were not significantly different from each other (Table 9). The cookie diameters of all the blends and control were significantly different from each other, and the diameters of the cookies baked from the 20% bran blend and 4.4% arabinoxylan blend were both less than the diameter of the cookies from the control white flour (Table 9). Polysaccharides such as cellulose and arabinoxylan are more rigid than amylose and amylopectin, and have been known to cause decrease in spread when added to cookie blends (James et al., 1989). Moreover, increased cookie moisture can decrease the glass transition temperature, which lowers cookie spread (Miller et al., 1997), and the presence of polysaccharides was associated with substantially increased cookie moisture in the current study (Table 7).

Table 9. Physical Qualities[1] of Cookies Baked from Different Blends of Ambassador Wheat Flour Fortified with Identical Amounts of Ferulic Acid (n=3)

Sample Number	Flour Blend	Cookie Quality		
		Single cookie diameter (cm)	Single cookie height (cm)	Hardness (N)
1	White flour (control)	7.78 ± 0.40a	0.98 ± 0.02b	22.00 ± 3.60c
2	20% Bran	7.44 ± 0.44b	1.18 ± 0.24a	27.60 ± 3.89b
3	4.4% Arabinoxylan	6.80 ± 0.16c	1.26 ± 0.18a	28.92 ± 3.86a
4	Free ferulic acid	8.01 ± 0.15d	0.92 ± 0.05b	28.69 ± 3.30a
5	20% Digested bran + free ferulic acid	8.30 ± 0.26e	0.88 ± 0.10b	24.78 ± 3.88d

[1] Values that do not share the same letter in the same column are significantly different from each other ($p<0.05$). Hardness was measured by the peak force of cookie breakage.

4.6.5. Cookie Hardness

The control cookies were the softest with 22 N peak force required to break the cookies, while the flour blend cookies with 4.4% arabinoxylan and with FA powder produced the hardest cookies, with both having 29 N peak force values (Table 9). The digested and non-digested bran cookies were each harder than cookies of the control white flour, but softer than those of the arabinoxylan and FA powder blends. Differences in baked product moisture did not significantly impact the hardness of the cookies, so "case hardening" from internal moisture relocation could not have been the likely cause of the difference in cookie hardness. The intermediate cookie hardness baked from flour blends with 20% digested (#2) or undigested bran could be due to the decreased starch content and increased cellulose content of those blends, which could have increased dough compactness (Gujral et al., 2003). The arabinoxylan blend cookies had high moisture content, yet the greatest hardness value, which indicates that water retention did not soften the cookies. This is in confirmation with other reports of cookies fortified with arabinoxylan oligosaccharides showing increased baked cookie hardness (Pareyt et al., 2011).

4.6.6. Changes in Total Ferulic Acid Contents after Baking

The total ferulic acid (FA) contents of all the cookie samples (in ground powder form) were measured using RP-HPLC as described in Chapter 3. The procedure used was the same as

in the digestion of whole ground flour, as ground cookie powders had well dispersed in the alkaline solution without complications. From the results obtained in this study, there were noticeable losses in the FA contents of the baked cookies made from all of the flour blends, including the losses in the naturally occurring bound ferulic acid in the undigested bran and arabinoxylan blends (Figure 9).

The blends (#2 through #5) all used flour milled from the Ambassador wheat variety. They were all formulated to the same FA content of 324 μg/g, which was based on the FA content of the ground whole ground flour from Ambassador. The undigested bran and arabinoxylan contained naturally occurring bound FA, and were added to the white flour proportionally so that the total FA content of the flour blend sample reached the same amount as that of ground whole ground flour from Ambassador. For all blends (except control), this translated to 180 μg FA/g of mixed cookie dough (Figure 9). The 20% bran flour blend had the highest FA content remaining after baking, at 155 μg FA/g ground baked cookie powder. This was a drop from the initial 180 μg FA/g, for a 90% retention rate. Cookies made from the flour blended with 20% digested bran fortified with free FA had the lowest amount of FA remaining, with 38% FA retention (Figures 9 and 10). The amount of FA remaining in the baked cookies made with the 4.4% arabinoxylan blend (sample #3) and the free FA blend (sample #4) were very similar, at 98 (54%) and 95 (53%) μg FA/g ground baked cookie powder, respectively. From the analysis of the data, there was no significant difference in the post-baking FA retention between samples #3 and #4, showing evidence of considerable amount of ferulic acid oxidation in both cookie blends from the massive decrease in measured ferulic acid content. The loss of ferulic acids during baking of the digested bran blend (sample #5) indicated that there was

more than a physical mechanism due to differences with the undigested bran sample, and it is highly likely that the polymerization of lignin-carbohydrate cross-linked complexes had a critical role in stabilizing ferulic acid by the incorporation into the lignin-network within the plant cell wall (Ralph et al., 1995), which may have been the cause for the preservation of the bound ferulic acid in the undigested bran cookie after baking (20% bran blend cookies vs. 20% digested bran with free FA blend cookies). The lack of similar FA preservation in the arabinoxylan blend despite also being in the bound form gives further evidence to the important role played by bran.

The free FA blend cookies showed identical amounts of FA loss as the arabinoxylan blend cookies, which will be elaborated further in Section 4.6.6. The similar light coloring of the cookies baked from both of these blends showed that the lost FA did not partake in browning reactions, which provides evidence of possible ferulic acid cross linkage with flour proteins. When ferulic acid is oxidized into quinones, it can chemically bond with the amino and thiol groups in proteins (Figueroa-Espinoza et al., 1999). The free radical of ferulic acid can also react with the tyrosine groups of proteins to form diferulic acid, which is a crosslinking agent for polysaccharide chains (Vansteenkiste et al., 2004). The addition of ferulic acid to flour in quantities similar to this study has been known to decrease gluten crosslinking and gluten elasticity (Koh and Ng, 2009), which would result in increased hardness and brittleness in low moisture baked products. The amount of ferulic acid remaining after baking had the highest Pearson correlation with cookie hardness out of all other cookie properties at 0.77 (Appendix A, Table 11).

Based on the results of this study, the addition of undigested natural bran to flour would be the most cost effective approach of ferulic acid fortification in the perspective of maximizing ferulic acid retention, as it requires the least chemical and physical processing other than size reduction of the bran particles. However, baking using digested bran has shown that the breakdown of bran structure aided in the degradation of ferulic acid and led to possible enzymatic browning of cookies. This means that any potential industrial application using wheat bran flour blends need to take bran stability and storage into consideration. The addition of arabinoxylan powder had shown no advantages over using free ferulic acid powder in decreasing ferulic acid loss during baking, and was the least cost-effective approach among the four different flour blends studied. With the most direct and cheapest fortification method by the addition of bran being the most effective, the fortification of ferulic acid in flour-based food products is absolutely technically feasible and economically viable, as long as the products are able to utilize whole ground flour and/or wheat bran. The proportion of bran added to the cookies was a moderate amount, meaning that the data of this study is directly applicable to commercial whole ground flour. For simplicity of the model, the shorts fraction was not added to the blend for testing in order to avoid confusion due to differences in the composition between bran and shorts.

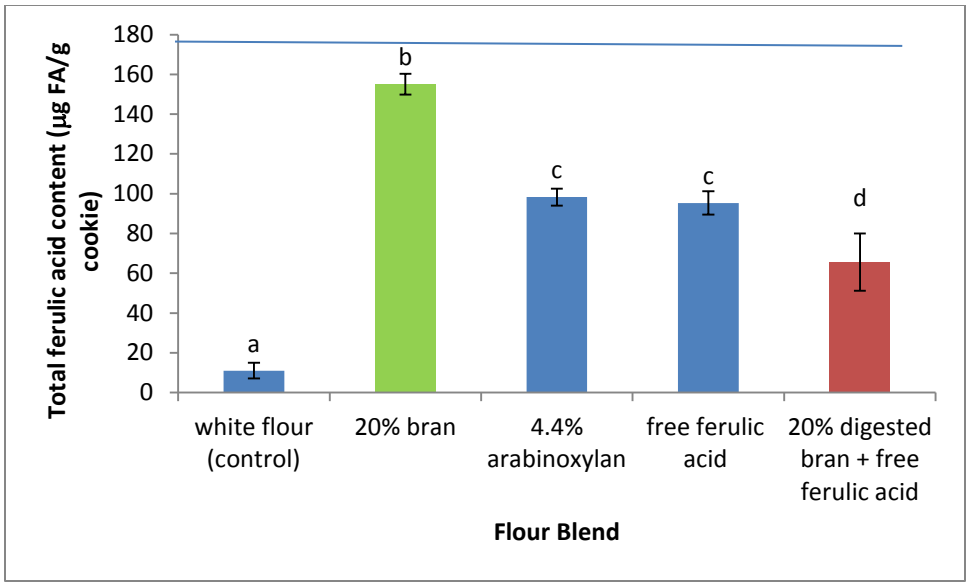

Figure 9. Total Ferulic Acid Content of Baked Cookie Samples made from Various White Flour Blends. Bars not sharing the same letter are significantly different from each other ($p<0.05$). The blue line is an indication of the initially fortified level measured in the cookie dough of the treatments before baking, excluding the original control flour. The ferulic acid content of the control cookie without fortification was included for reference.

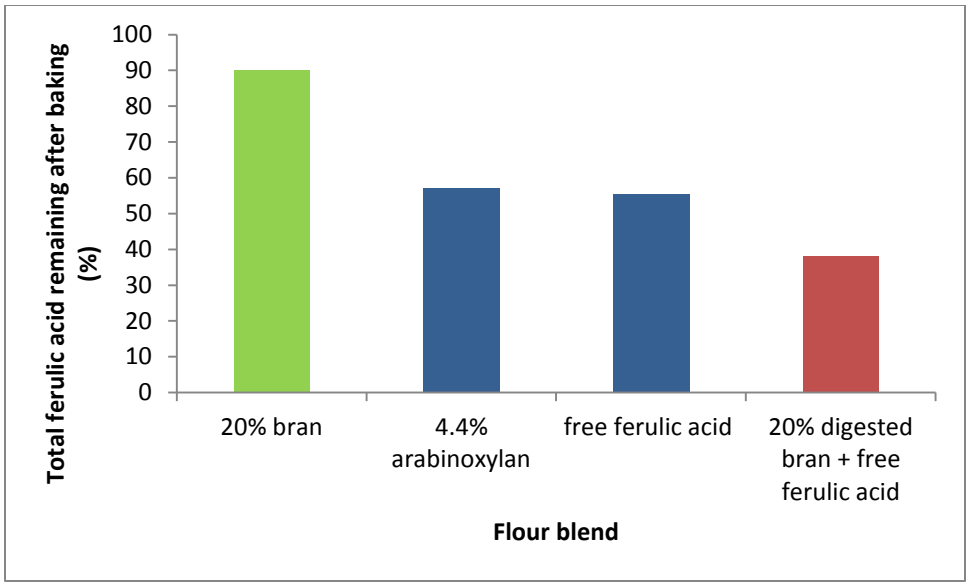

Figure 10. Percent of Ferulic Acid Retained in Cookies after Baking Cookie Doughs made from Four Different White Flour Blends of Wheat Variety Ambassador.

CHAPTER 5

CONCLUSIONS AND FUTURE RECOMMENDATIONS

During the first experiments of this thesis, the viability of enzymatic digestion versus alkaline digestion of ground whole ground wheat flour was compared, for the purpose of ferulic acid extraction and quantification. Although the enzymatic digestion method initially showed promise in extracting substantial amounts of ferulic acid, the sheer amount of enzymes required to reach the highest optimal extraction time made the procedure financially unfeasible for use in experiments involving large numbers of sample materials. The time of the entire enzymatic digestion process was longer than the alkaline digestion method with a more complex sample preparation step. By taking these factors into consideration, the enzymatic digestion method was deemed inefficient for the scope of the experiments for the current study, and its potential use was discarded in favor of the alkaline digestion method for the extraction of total phenolic contents and ferulic acid contents from the ground wheat flour.

After the total phenolic contents and ferulic acid contents of the selected Michigan wheat varieties were quantified, the results showed a general increase in detectable phenolic acids from wheat varieties of the 2014 harvest compared with those of the 2013 harvest. In addition, the number of significantly different variety groupings decreased in the 2014 harvest season. Using the data obtained from the Michigan Enviro-Weather Program, the most likely environmental factor causing the overall increase in phenolic acid synthesis in the crops was the severe cold winter of the 2014 season. In future experiments, growing wheat samples in controlled temperatures and deliberately exposing them to consistent lower air temperature

may help in detecting changes in gene expression and identify the genes responsible for the increased phenolic acid synthesis. This could in turn assist in the breeding of potential new wheat varieties with the purpose of yielding increased or decreased phenolic acid contents.

From the cookie baking experiments conducted with different cookie flour blends fortified with ferulic acid, the antioxidant retention was highest in the flour fortified with bran containing bound ferulic acid. The cookies showing the least amount of changes in baking quality were made from the flour blend with added free ferulic acid powder. This type of cookie had the least amount of differences in physical appearances, such as color and dimension, but had significantly the greatest losses in ferulic acid upon baking. Aside from the digested bran flour blend with released polyphenol oxidase, the addition of ferulic acid to the wheat flour generally increased baked cookie lightness. The water retention ability of the bran and arabinoxylan affected cookie hardness, spread and height; ferulic acid oxidation-induced protein cross-linkage was also another possible factor in increasing cookie hardness. Of the four blends studied, the bran flour blend was the most cost effective and had the highest ferulic acid retention levels after cookie baking. In future experiments, baking with modifications of the cookie formulation could be performed in order to minimize differences in cookie quality between the bran flour blend and the control white flour blend. This could involve changes to the amount of any number of the wet and dry ingredients, such as flour, water, shortening, brown and white sugars, corn syrup, and ammonium bicarbonate.

APPENDICES

APPENDIX A. Supplementary Data for Wheat Phenolic Quantification and Cookie Baking

Table 10. Preparation of Ferulic Acid Standard Solution for RP-HPLC Analysis

Tube #	Ferulic acid conc. (ppm)	100ppm Ferulic acid solution volume (ml)	Methanol volume to pipette (ml)
1	10	0.5	4.5
2	20	1.0	4.0
3	30	1.5	3.5
4	40	2.0	3
5	50	2.5	2.5
6	60	3.0	2
7	70	3.5	1.5
8	80	4.0	1
9	90	4.5	.5
10	100	5.0	0

Table 11. Pearson Product-moment Correlation Coefficient between Different Parameters from Cookie Baking

	L*	a*	b*	Cookie moisture content	Ferulic acid content after baking	Cookie diameter	Cookie height	Cookie hardness
Cookie moisture content	0.76	-0.89	0.11	NA	0.70	-0.90	0.98	0.56
Ferulic acid content after baking	0.38	-0.41	0.07	0.70	NA	-0.39	0.56	0.77
Cookie treatment	Post-baking final ferulic acid content (μg FA/g)	Standard deviation						
white flour	10.91	±3.97						
20% bran	155.03	±5.22						
4.4% arabinoxylan	98.32	±4.27						
free ferulic acid	95.36	±5.81						
20% digested bran + free ferulic acid	65.63	±14.40						

Table 12. Significant Differences of Total Populations Measured using One way ANOVA with Fisher Comparison. Populations with $p<0.05$ were deemed significantly different

Population parameter	F value	P value
2013 TPC	18.920	<0.001
2013 bound PC	10.430	<0.001
2013 free PC	1.200	0.319
2013 total FA	10.830	<0.001
2013 bound FA	41.970	<0.001
2013 free FA	6.270	<0.001
2014 TPC	1.020	<0.001
2014 bound PC	0.830	0.009
2014 free PC	0.890	0.001
2014 total FA	2.050	0.014
2014 bound FA	1.920	0.023
2014 free FA	1.620	0.069
Cookie lightness (L*)	62.220	<0.001
Cookie color (a*)	8.100	0.001
Cookie color (b*)	21.320	<0.001
Cookie diameter	52.260	<0.001
Cookie height	20.650	<0.001
Cookie hardness	2.530	0.084
Cookie moisture content	351.570	<0.001
Cookie total FA content after baking	91.370	<0.001

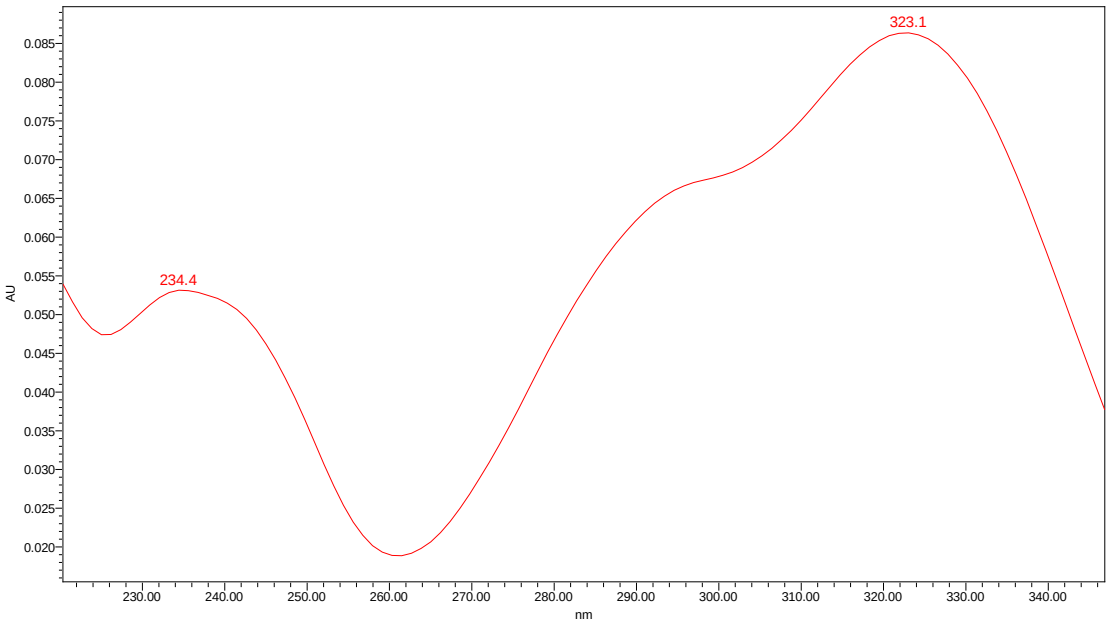

Figure 11. UV-Visible Spectrum of Ferulic Acid Extracted from Michigan Soft Wheat Whole Ground Flour (Ambassador).

APPENDIX B. Viability of Enzymatic Digestion of Wheat Flour with Feruloyl Esterase

Testing of the Ambassador variety flour with different enzyme combinations without a 30-minute heat pre-treatment of the samples on a hot plate at 100^{o}C did not yield any significant ferulic acid release, except for small amounts of free phenolic acids (Appendix B, Figure 12). Flour samples that were given heat pre-treatment before the introduction of enzymes showed greatly increased ferulic acid (FA) measurements (Appendix B, Figure 13), indicating interaction of the added enzymes with the gelatinized starch constituents and the subsequent release of ferulic acid.

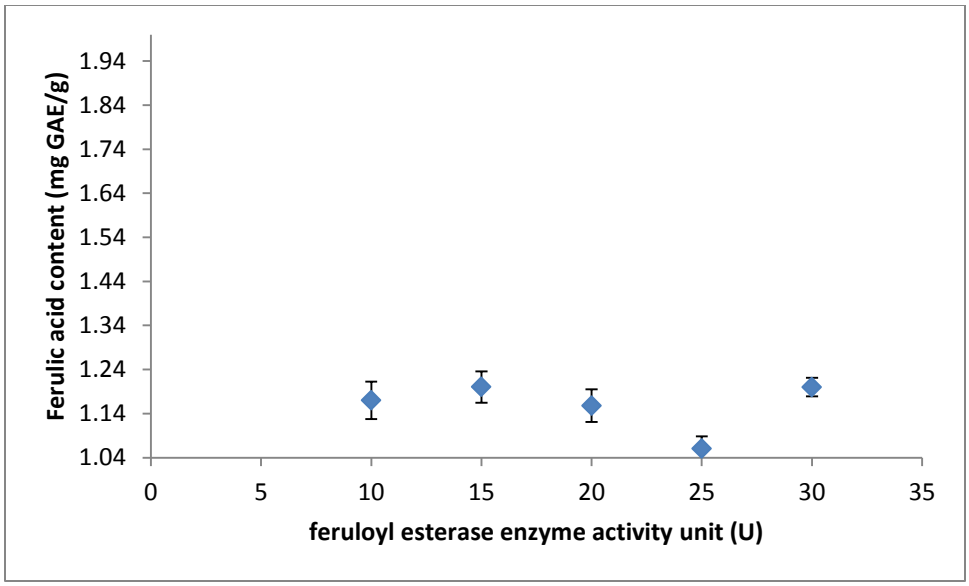

Figure 12. Ferulic Content of Wheat Samples Treated with Constant Xylanase at 20 U and increasing amounts of Feruloyl Esterase (see Table 2, without pre-heating treatment).

The increase in the amount of enzymes added to the flour samples corresponded to the release of ferulic acid (Appendix B, Figure 14). The treatment with increasing levels of feruloyl esterase (FE) correlated with a gradual increase in measured FA while treatment with increasing levels of xylanase released higher amounts of phenolic acids at lower concentrations (Appendix B, Figure 13, 14). From results, the experiment showed that a proportionally smaller amount of xylanase (5 U) was required for the optimal release of ferulic acid from the flour when compared to amount of FE required at 20 U (Appendix B, Figure 13, 14).

The TPC measurement of the representative variety Ambassador in the current experiment was 3.1 mg/g Gallic Acid Equivalents (GAE). The Ambassador sample colorimetric measurement by alkaline extraction after defatting and washing with ethyl acetate was 3.5mg/g GAE. The proportion of the TPC from enzymatic digestion in comparison to that from alkaline digestion is reasonable, as other phenolic acids were not extracted by the enzymatic method.

Overall, the total time for sample preparation, digestion, and collection for enzymatic digestion was approximately 5 hours, compared to 4 hours for the alkaline digestion. However, the amount of enzymes required for optimal digestion was very high. This method was not cost effective for extracting ferulic acid from large numbers of different samples, and it was not economically feasible to employ this method for all of the wheat varieties for the rest of the experiments of this thesis. However, the enzymatic digestion method can still be useful for certain situations where isolation of ferulic acid and minimizing the presence of other starch constituents would be desired.

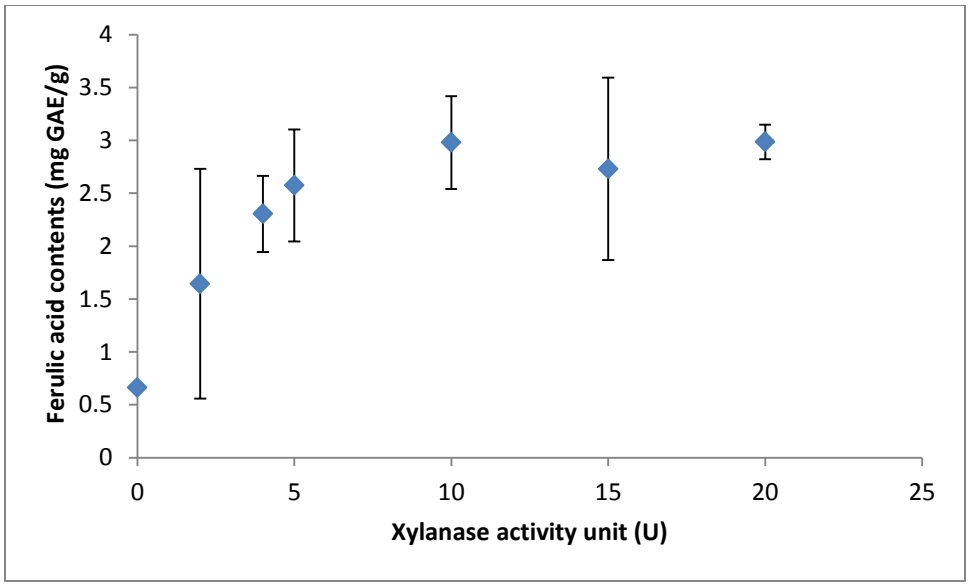

Figure 13. Ferulic Acid Content of Wheat Samples Treated with Constant FE at 20 U and Increasing Concentrations of Xyl (Table 2, with heating).

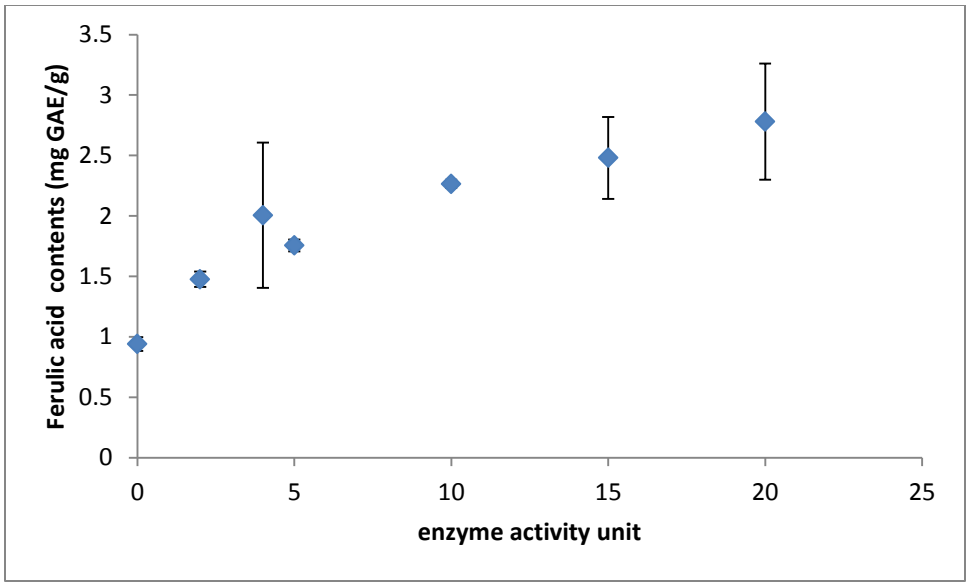

Figure 14. Ferulic Acid Content of Wheat Samples Treated With Constant Levels of Xyl at 20 U, and Increasing Additions of FE, With Heat Pre-Treatment[1].

[1] *For details, see Table 2.*

APPENDIX C. Statistical Analysis Raw Data

2013 Factor Information

```
Factor  Levels  Values
C1          57  E0028, E1007R, E5011, F1003R, F1012, F1014, F1026R, F1027,
F1029, F1032R,
                F1047, F1048, F1049, F1050, F1051, F2001R, F2002, F2003,
F2004, F2005, F2006,
                F2008, F2009, F2011, F2012, F2014R, F2015, F2016, F2018,
F2019, F2020, F2021,
                F2022, F2024R, F2025R, F2027R, F2028R, F2029R, F2030, F2031,
F2032, F2033,
                F2034, F2035, F2036, F2037, F2038, F2039, F2040, F2041,
F2042, I5440, I7067,
                I7127, I7826, I9339, I9340
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2014 Factor Information

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Factor  Levels  Values
C1          39  E0028, E5011, F0013R, F1026R, F1027, F1029, F1047, F1048,
F1049, F2002,
                F2003, F2005, F2008, F2009, F2010, F2012, F2014R, F2015,
F2016, F2018, F2019,
                F2020, F2021, F2022, F2024R, F2028R, F2029R, F2030, F2031,
F2033, F2034,
                F2037, F2038, F2039, F2042, I7826, x1, x2, x3
```

Table 13.1. Raw Data for 2013 Total Ferulic Acid Content

One-way ANOVA: C2 versus C1

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	56	0.17683	0.003158	10.95	0.000
Error	57	0.01643	0.000288		
Total	113	0.19327			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0169801	91.50%	83.14%	65.99%

Means

C1	N	Mean	StDev	95% CI
Ambassador	2	0.35762	0.01308	(0.33358, 0.38167)
Aubrey	2	0.31268	0.01227	(0.28864, 0.33672)
Caledonia	2	0.32529	0.00906	(0.30124, 0.34933)
Cayuga	2	0.33490	0.01274	(0.31086, 0.35894)
Hopewell	2	0.3579	0.0247	(0.3339, 0.3819)
Jupiter	2	0.3379	0.0337	(0.3139, 0.3620)
MSU Line F1003R	2	0.24959	0.00931	(0.22555, 0.27363)
MSU Line F1012	2	0.3103	0.0219	(0.2862, 0.3343)
MSU Line F1014	2	0.36218	0.00562	(0.33813, 0.38622)
MSU Line F1026R	2	0.30223	0.00551	(0.27818, 0.32627)
MSU Line F1027	2	0.35091	0.00156	(0.32687, 0.37495)
MSU Line F1029	2	0.32584	0.00718	(0.30179, 0.34988)
MSU Line F1032R	2	0.30975	0.00312	(0.28571, 0.33379)
MSU Line F1047	2	0.2882	0.0162	(0.2642, 0.3123)
MSU Line F1048	2	0.3376	0.0268	(0.3136, 0.3617)
MSU Line F1049	2	0.30909	0.00859	(0.28505, 0.33314)
MSU Line F1050	2	0.35835	0.01303	(0.33431, 0.38239)
MSU Line F1051	2	0.261759	0.001143	(0.237716, 0.285802)
MSU Line F2001R	2	0.3261	0.0325	(0.3020, 0.3501)
MSU Line F2002	2	0.31799	0.00807	(0.29395, 0.34203)
MSU Line F2003	2	0.39026	0.00523	(0.36622, 0.41430)
MSU Line F2004	2	0.3557	0.0184	(0.3316, 0.3797)

Table 13.1 (cont'd)

MSU Line F2005	2	0.31841	0.00328	(0.29437,	0.34245)
MSU Line F2006	2	0.23554	0.01211	(0.21150,	0.25958)
MSU Line F2008	2	0.33907	0.00400	(0.31503,	0.36311)
MSU Line F2009	2	0.32160	0.00726	(0.29756,	0.34565)
MSU Line F2011	2	0.3659	0.0258	(0.3418,	0.3899)
MSU Line F2012	2	0.2853	0.0407	(0.2612,	0.3093)
MSU Line F2014R	2	0.28355	0.01164	(0.25951,	0.30760)
MSU Line F2015	2	0.37037	0.01172	(0.34632,	0.39441)
MSU Line F2016	2	0.2896	0.0258	(0.2656,	0.3137)
MSU Line F2018	2	0.36565	0.00972	(0.34161,	0.38969)
MSU Line F2019	2	0.2857	0.0147	(0.2616,	0.3097)
MSU Line F2020	2	0.2933	0.0223	(0.2693,	0.3174)
MSU Line F2021	2	0.3105	0.0398	(0.2865,	0.3346)
MSU Line F2022	2	0.39717	0.01052	(0.37313,	0.42121)
MSU Line F2024R	2	0.265141	0.001062	(0.241098,	0.289184)
MSU Line F2025R	2	0.290139	0.000458	(0.266096,	0.314182)
MSU Line F2027R	2	0.33946	0.00334	(0.31542,	0.36350)
MSU Line F2028R	2	0.32588	0.00991	(0.30184,	0.34992)
MSU Line F2029R	2	0.3434	0.0207	(0.3194,	0.3675)
MSU Line F2030	2	0.3032	0.0216	(0.2792,	0.3273)
MSU Line F2031	2	0.31337	0.00342	(0.28933,	0.33741)
MSU Line F2032	2	0.3737	0.0224	(0.3496,	0.3977)
MSU Line F2033	2	0.27257	0.00316	(0.24853,	0.29661)
MSU Line F2034	2	0.293135	0.001014	(0.269092,	0.317178)
MSU Line F2035	2	0.3602	0.0429	(0.3361,	0.3842)
MSU Line F2036	2	0.25297	0.01131	(0.22892,	0.27701)
MSU Line F2037	2	0.31466	0.00151	(0.29062,	0.33871)
MSU Line F2038	2	0.29723	0.00180	(0.27319,	0.32127)
MSU Line F2039	2	0.412480	0.001059	(0.388437,	0.436524)
MSU Line F2040	2	0.25763	0.01169	(0.23359,	0.28168)
MSU Line F2041	2	0.34169	0.01260	(0.31764,	0.36573)
MSU Line F2042	2	0.3711	0.0148	(0.3471,	0.3952)
Red Ruby	2	0.3298	0.0148	(0.3058,	0.3538)
VA09W-188WS	2	0.27280	0.01176	(0.24876,	0.29685)
VA09W-192WS	2	0.2654	0.0186	(0.2413,	0.2894)

Pooled StDev = 0.0169801

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

C1	N	Mean	Grouping
MSU Line F2039	2	0.412480	A
MSU Line F2022	2	0.39717	A B
MSU Line F2003	2	0.39026	A B C
MSU Line F2032	2	0.3737	B C D
MSU Line F2042	2	0.3711	B C D E
MSU Line F2015	2	0.37037	B C D E
MSU Line F2011	2	0.3659	B C D E F
MSU Line F2018	2	0.36565	B C D E F

Table 13.1 (cont'd)

MSU Line F1014	2	0.36218	C D E F G
MSU Line F2035	2	0.3602	C D E F G
MSU Line F1050	2	0.35835	C D E F G H
Hopewell	2	0.3579	C D E F G H
Ambassador	2	0.35762	C D E F G H
MSU Line F2004	2	0.3557	D E F G H
MSU Line F1027	2	0.35091	D E F G H I
MSU Line F2029R	2	0.3434	D E F G H I J
MSU Line F2041	2	0.34169	D E F G H I J K
MSU Line F2027R	2	0.33946	E F G H I J K
MSU Line F2008	2	0.33907	E F G H I J K
Jupiter	2	0.3379	E F G H I J K
MSU Line F1048	2	0.3376	E F G H I J K
Cayuga	2	0.33490	F G H I J K L
Red Ruby	2	0.3298	G H I J K L M
MSU Line F2001R	2	0.3261	H I J K L M N
MSU Line F2028R	2	0.32588	H I J K L M N
MSU Line F1029	2	0.32584	H I J K L M N
Caledonia	2	0.32529	H I J K L M N
MSU Line F2009	2	0.32160	I J K L M N O
MSU Line F2005	2	0.31841	I J K L M N O P
MSU Line F2002	2	0.31799	I J K L M N O P
MSU Line F2037	2	0.31466	J K L M N O P Q
MSU Line F2031	2	0.31337	J K L M N O P Q
Aubrey	2	0.31268	J K L M N O P Q
MSU Line F2021	2	0.3105	J K L M N O P Q
MSU Line F1012	2	0.3103	J K L M N O P Q
MSU Line F1032R	2	0.30975	J K L M N O P Q
MSU Line F1049	2	0.30909	K L M N O P Q
MSU Line F2030	2	0.3032	L M N O P Q R
MSU Line F1026R	2	0.30223	L M N O P Q R
MSU Line F2038	2	0.29723	M N O P Q R S
MSU Line F2020	2	0.2933	N O P Q R S T
MSU Line F2034	2	0.293135	N O P Q R S T
MSU Line F2025R	2	0.290139	O P Q R S T U
MSU Line F2016	2	0.2896	O P Q R S T U
MSU Line F1047	2	0.2882	O P Q R S T U
MSU Line F2019	2	0.2857	P Q R S T U V
MSU Line F2012	2	0.2853	P Q R S T U V
MSU Line F2014R	2	0.28355	Q R S T U V W
VA09W-188WS	2	0.27280	R S T U V W
MSU Line F2033	2	0.27257	R S T U V W
VA09W-192WS	2	0.2654	S T U V W X
MSU Line F2024R	2	0.265141	S T U V W X
MSU Line F1051	2	0.261759	T U V W X
MSU Line F2040	2	0.25763	U V W X
MSU Line F2036	2	0.25297	V W X
MSU Line F1003R	2	0.24959	W X
MSU Line F2006	2	0.23554	X

Means that do not share a letter are significantly different.

Table 13.2. Raw Data for 2013 Bound Ferulic Acid Content

One-way ANOVA: C2 versus C1

```
* ERROR * Cannot draw the interval plot. Interval plots are illegible with
more than 45
          intervals.

Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.

Analysis of Variance

Source   DF  Adj SS  Adj MS  F-Value  P-Value
C1       56  176833  3157.7    10.95    0.000
Error    57   16434   288.3
Total   113  193268

Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
16.9801  91.50%     83.14%      65.99%

Means

C1               N     Mean  StDev        95% CI
Ambassador       2   357.62  13.08  ( 333.58,  381.67)
Aubrey           2   312.68  12.27  ( 288.64,  336.72)
Caledonia        2   325.29   9.06  ( 301.24,  349.33)
Cayuga           2   334.90  12.74  ( 310.86,  358.94)
Hopewell         2    357.9   24.7  (  333.9,   381.9)
Jupiter          2    337.9   33.7  (  313.9,   362.0)
MSU Line F1003R  2   249.59   9.31  ( 225.55,  273.63)
MSU Line F1012   2    310.3   21.9  (  286.2,   334.3)
MSU Line F1014   2   362.18   5.62  ( 338.13,  386.22)
MSU Line F1026R  2   302.23   5.51  ( 278.18,  326.27)
MSU Line F1027   2   350.91   1.56  ( 326.87,  374.95)
MSU Line F1029   2   325.84   7.18  ( 301.79,  349.88)
MSU Line F1032R  2   309.75   3.12  ( 285.71,  333.79)
MSU Line F1047   2    288.2   16.2  (  264.2,   312.3)
MSU Line F1048   2    337.6   26.8  (  313.6,   361.7)



MSU Line F1049   2   309.09   8.59  ( 285.05,  333.14)
MSU Line F1050   2   358.35  13.03  ( 334.31,  382.39)
MSU Line F1051   2  261.759  1.143  (237.716, 285.802)
MSU Line F2001R  2    326.1   32.5  (  302.0,   350.1)
MSU Line F2002   2   317.99   8.07  ( 293.95,  342.03)
MSU Line F2003   2   390.26   5.23  ( 366.22,  414.30)
```

Table 13.2 (cont'd)

```
MSU Line F2004   2    355.7   18.4  (  331.6,   379.7)
MSU Line F2005   2   318.41   3.28  ( 294.37,  342.45)
```

MSU Line F2006	2	235.54	12.11	(211.50,	259.58)
MSU Line F2008	2	339.07	4.00	(315.03,	363.11)
MSU Line F2009	2	321.60	7.26	(297.56,	345.65)
MSU Line F2011	2	365.9	25.8	(341.8,	389.9)
MSU Line F2012	2	285.3	40.7	(261.2,	309.3)
MSU Line F2014R	2	283.55	11.64	(259.51,	307.60)
MSU Line F2015	2	370.37	11.72	(346.32,	394.41)
MSU Line F2016	2	289.6	25.8	(265.6,	313.7)
MSU Line F2018	2	365.65	9.72	(341.61,	389.69)
MSU Line F2019	2	285.7	14.7	(261.6,	309.7)
MSU Line F2020	2	293.3	22.3	(269.3,	317.4)
MSU Line F2021	2	310.5	39.8	(286.5,	334.6)
MSU Line F2022	2	397.17	10.52	(373.13,	421.21)
MSU Line F2024R	2	265.141	1.062	(241.098,	289.184)
MSU Line F2025R	2	290.139	0.458	(266.096,	314.182)
MSU Line F2027R	2	339.46	3.34	(315.42,	363.50)
MSU Line F2028R	2	325.88	9.91	(301.84,	349.92)
MSU Line F2029R	2	343.4	20.7	(319.4,	367.5)
MSU Line F2030	2	303.2	21.6	(279.2,	327.3)
MSU Line F2031	2	313.37	3.42	(289.33,	337.41)
MSU Line F2032	2	373.7	22.4	(349.6,	397.7)
MSU Line F2033	2	272.57	3.16	(248.53,	296.61)
MSU Line F2034	2	293.135	1.014	(269.092,	317.178)
MSU Line F2035	2	360.2	42.9	(336.1,	384.2)
MSU Line F2036	2	252.97	11.31	(228.92,	277.01)
MSU Line F2037	2	314.66	1.51	(290.62,	338.71)
MSU Line F2038	2	297.23	1.80	(273.19,	321.27)
MSU Line F2039	2	412.480	1.059	(388.437,	436.524)
MSU Line F2040	2	257.63	11.69	(233.59,	281.68)
MSU Line F2041	2	341.69	12.60	(317.64,	365.73)
MSU Line F2042	2	371.1	14.8	(347.1,	395.2)
Red Ruby	2	329.8	14.8	(305.8,	353.8)
VA09W-188WS	2	272.80	11.76	(248.76,	296.85)
VA09W-192WS	2	265.4	18.6	(241.3,	289.4)

Pooled StDev = 16.9801

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

C1	N	Mean	Grouping
MSU Line F2039	2	412.480	A
MSU Line F2022	2	397.17	A B
MSU Line F2003	2	390.26	A B C
MSU Line F2032	2	373.7	B C D
MSU Line F2042	2	371.1	B C D E
MSU Line F2015	2	370.37	B C D E
MSU Line F2011	2	365.9	B C D E F
MSU Line F2018	2	365.65	B C D E F

Table 13.2 (cont'd)

MSU Line F1014	2	362.18	C D E F G
MSU Line F2035	2	360.2	C D E F G
MSU Line F1050	2	358.35	C D E F G H
Hopewell	2	357.9	C D E F G H

Ambassador	2	357.62	C D E F G H
MSU Line F2004	2	355.7	D E F G H
MSU Line F1027	2	350.91	D E F G H I
MSU Line F2029R	2	343.4	D E F G H I J
MSU Line F2041	2	341.69	D E F G H I J K
MSU Line F2027R	2	339.46	E F G H I J K
MSU Line F2008	2	339.07	E F G H I J K
Jupiter	2	337.9	E F G H I J K
MSU Line F1048	2	337.6	E F G H I J K
Cayuga	2	334.90	F G H I J K L
Red Ruby	2	329.8	G H I J K L M
MSU Line F2001R	2	326.1	H I J K L M N
MSU Line F2028R	2	325.88	H I J K L M N
MSU Line F1029	2	325.84	H I J K L M N
Caledonia	2	325.29	H I J K L M N
MSU Line F2009	2	321.60	I J K L M N O
MSU Line F2005	2	318.41	I J K L M N O P
MSU Line F2002	2	317.99	I J K L M N O P
MSU Line F2037	2	314.66	J K L M N O P Q
MSU Line F2031	2	313.37	J K L M N O P Q
Aubrey	2	312.68	J K L M N O P Q
MSU Line F2021	2	310.5	J K L M N O P Q
MSU Line F1012	2	310.3	J K L M N O P Q
MSU Line F1032R	2	309.75	J K L M N O P Q
MSU Line F1049	2	309.09	K L M N O P Q
MSU Line F2030	2	303.2	L M N O P Q R
MSU Line F1026R	2	302.23	L M N O P Q R
MSU Line F2038	2	297.23	M N O P Q R S
MSU Line F2020	2	293.3	N O P Q R S T
MSU Line F2034	2	293.135	N O P Q R S T
MSU Line F2025R	2	290.139	O P Q R S T U
MSU Line F2016	2	289.6	O P Q R S T U
MSU Line F1047	2	288.2	O P Q R S T U
MSU Line F2019	2	285.7	P Q R S T U V
MSU Line F2012	2	285.3	P Q R S T U V
MSU Line F2014R	2	283.55	Q R S T U V W
VA09W-188WS	2	272.80	R S T U V W
MSU Line F2033	2	272.57	R S T U V W
VA09W-192WS	2	265.4	S T U V W X
MSU Line F2024R	2	265.141	S T U V W X
MSU Line F1051	2	261.759	T U V W X
MSU Line F2040	2	257.63	U V W X
MSU Line F2036	2	252.97	V W X
MSU Line F1003R	2	249.59	W X
MSU Line F2006	2	235.54	X

Means that do not share a letter are significantly different.

Table 13.3. Raw Data for 2013 Free Ferulic Acid Content

One-way ANOVA: C2 versus C1

```
* ERROR * Cannot draw the interval plot. Interval plots are illegible with
more than 45
          intervals.

Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Analysis of Variance

Source   DF  Adj SS  Adj MS  F-Value  P-Value
C1       56  3251.3   58.06     3.75    0.000
Error    57   881.4   15.46
Total   113  4132.8

Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
3.93240  78.67%     57.72%      14.69%


Means

C1               N    Mean  StDev       95% CI
Ambassador       2   21.40   2.83  ( 15.83,  26.97)
Aubrey           2   16.20   2.12  ( 10.63,  21.77)
Caledonia        2   14.10   2.12  (  8.53,  19.67)
Cayuga           2   19.40   2.83  ( 13.83,  24.97)
Hopewell         2   20.80   4.95  ( 15.23,  26.37)
Jupiter          2   19.70   7.07  ( 14.13,  25.27)
MSU Line F1003R  2   18.40   2.83  ( 12.83,  23.97)
MSU Line F1012   2   17.00   4.95  ( 11.43,  22.57)
MSU Line F1014   2   13.90   3.54  (  8.33,  19.47)
MSU Line F1026R  2   13.50   1.41  (  7.93,  19.07)
MSU Line F1027   2  13.800  0.283  ( 8.232, 19.368)
MSU Line F1029   2   13.40   1.41  (  7.83,  18.97)
MSU Line F1032R  2  11.900  0.707  ( 6.332, 17.468)
MSU Line F1047   2   17.40   4.24  ( 11.83,  22.97)
MSU Line F1048   2   27.50   5.66  ( 21.93,  33.07)
MSU Line F1049   2   12.50   2.12  (  6.93,  18.07)
MSU Line F1050   2   32.10   2.83  ( 26.53,  37.67)
MSU Line F1051   2  21.700  0.283  (16.132, 27.268)
MSU Line F2001R  2   23.50   7.07  ( 17.93,  29.07)
MSU Line F2002   2   17.40   2.12  ( 11.83,  22.97)
MSU Line F2003   2  16.300  0.707  (10.732, 21.868)
MSU Line F2004   2   26.00   3.54  ( 20.43,  31.57)
```

Table 13.3 (cont'd)

MSU Line F2005	2	25.700	0.707	(20.132, 31.268)
MSU Line F2006	2	21.90	3.54	(16.33, 27.47)
MSU Line F2008	2	33.00	7.78	(27.43, 38.57)
MSU Line F2009	2	18.50	1.41	(12.93, 24.07)
MSU Line F2011	2	20.90	4.95	(15.33, 26.47)
MSU Line F2012	2	19.90	9.90	(14.33, 25.47)
MSU Line F2014R	2	18.40	2.83	(12.83, 23.97)
MSU Line F2015	2	16.90	2.12	(11.33, 22.47)
MSU Line F2016	2	19.10	6.36	(13.53, 24.67)
MSU Line F2018	2	26.550	0.212	(20.982, 32.118)
MSU Line F2019	2	14.00	3.54	(8.43, 19.57)
MSU Line F2020	2	12.00	4.95	(6.43, 17.57)
MSU Line F2021	2	26.80	9.19	(21.23, 32.37)
MSU Line F2022	2	15.20	2.12	(9.63, 20.77)
MSU Line F2024R	2	15.850	0.354	(10.282, 21.418)
MSU Line F2025R	2	18.600	0.141	(13.032, 24.168)
MSU Line F2027R	2	20.000	0.707	(14.432, 25.568)
MSU Line F2028R	2	20.20	2.12	(14.63, 25.77)
MSU Line F2029R	2	29.80	4.24	(24.23, 35.37)
MSU Line F2030	2	25.00	4.95	(19.43, 30.57)
MSU Line F2031	2	14.100	0.707	(8.532, 19.668)
MSU Line F2032	2	18.60	4.24	(13.03, 24.17)
MSU Line F2033	2	26.200	0.707	(20.632, 31.768)
MSU Line F2034	2	11.700	0.283	(6.132, 17.268)
MSU Line F2035	2	18.50	8.49	(12.93, 24.07)
MSU Line F2036	2	15.10	3.54	(9.53, 20.67)
MSU Line F2037	2	19.900	0.424	(14.332, 25.468)
MSU Line F2038	2	11.950	0.495	(6.382, 17.518)
MSU Line F2039	2	12.900	0.283	(7.332, 18.468)
MSU Line F2040	2	22.00	3.54	(16.43, 27.57)
MSU Line F2041	2	23.50	2.83	(17.93, 29.07)
MSU Line F2042	2	27.60	2.83	(22.03, 33.17)
Red Ruby	2	27.80	3.54	(22.23, 33.37)
VA09W-188WS	2	16.60	2.83	(11.03, 22.17)
VA09W-192WS	2	17.20	4.95	(11.63, 22.77)

Pooled StDev = 3.93240

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

C1	N	Mean	Grouping
MSU Line F2008	2	33.00	A
MSU Line F1050	2	32.10	A B
MSU Line F2029R	2	29.80	A B C
Red Ruby	2	27.80	A B C D
MSU Line F2042	2	27.60	A B C D E
MSU Line F1048	2	27.50	A B C D E F
MSU Line F2021	2	26.80	A B C D E F G
MSU Line F2018	2	26.550	A B C D E F G

Table 13.3 (cont'd)

MSU Line F2033	2	26.200	A	B	C	D	E	F	G	H															
MSU Line F2004	2	26.00	A	B	C	D	E	F	G	H															
MSU Line F2005	2	25.700	A	B	C	D	E	F	G	H															
MSU Line F2030	2	25.00		B	C	D	E	F	G	H	I														
MSU Line F2041	2	23.50			C	D	E	F	G	H	I	J													
MSU Line F2001R	2	23.50			C	D	E	F	G	H	I	J													
MSU Line F2040	2	22.00			C	D	E	F	G	H	I	J	K												
MSU Line F2006	2	21.90				D	E	F	G	H	I	J	K	L											
MSU Line F1051	2	21.700				D	E	F	G	H	I	J	K	L	M										
Ambassador	2	21.40				D	E	F	G	H	I	J	K	L	M	N									
MSU Line F2011	2	20.90				D	E	F	G	H	I	J	K	L	M	N	O								
Hopewell	2	20.80				D	E	F	G	H	I	J	K	L	M	N	O								
MSU Line F2028R	2	20.20				D	E	F	G	H	I	J	K	L	M	N	O	P							
MSU Line F2027R	2	20.000				D	E	F	G	H	I	J	K	L	M	N	O	P							
MSU Line F2037	2	19.900					E	F	G	H	I	J	K	L	M	N	O	P							
MSU Line F2012	2	19.90					E	F	G	H	I	J	K	L	M	N	O	P							
Jupiter	2	19.70						F	G	H	I	J	K	L	M	N	O	P	Q						
Cayuga	2	19.40							G	H	I	J	K	L	M	N	O	P	Q	R					
MSU Line F2016	2	19.10							G	H	I	J	K	L	M	N	O	P	Q	R					
MSU Line F2032	2	18.60								H	I	J	K	L	M	N	O	P	Q	R					
MSU Line F2025R	2	18.600								H	I	J	K	L	M	N	O	P	Q	R					
MSU Line F2035	2	18.50								H	I	J	K	L	M	N	O	P	Q	R					
MSU Line F2009	2	18.50								H	I	J	K	L	M	N	O	P	Q	R					
MSU Line F2014R	2	18.40								H	I	J	K	L	M	N	O	P	Q	R					
MSU Line F1003R	2	18.40								H	I	J	K	L	M	N	O	P	Q	R					
MSU Line F2002	2	17.40									I	J	K	L	M	N	O	P	Q	R					
MSU Line F1047	2	17.40									I	J	K	L	M	N	O	P	Q	R					
VA09W-192WS	2	17.20									I	J	K	L	M	N	O	P	Q	R					
MSU Line F1012	2	17.00										J	K	L	M	N	O	P	Q	R					
MSU Line F2015	2	16.90										J	K	L	M	N	O	P	Q	R					
VA09W-188WS	2	16.60										J	K	L	M	N	O	P	Q	R					
MSU Line F2003	2	16.300										J	K	L	M	N	O	P	Q	R					
Aubrey	2	16.20										J	K	L	M	N	O	P	Q	R					
MSU Line F2024R	2	15.850										J	K	L	M	N	O	P	Q	R					
MSU Line F2022	2	15.20											K	L	M	N	O	P	Q	R					
MSU Line F2036	2	15.10											K	L	M	N	O	P	Q	R					
MSU Line F2031	2	14.100												L	M	N	O	P	Q	R					
Caledonia	2	14.10												L	M	N	O	P	Q	R					
MSU Line F2019	2	14.00													M	N	O	P	Q	R					
MSU Line F1014	2	13.90													M	N	O	P	Q	R					
MSU Line F1027	2	13.800														N	O	P	Q	R					
MSU Line F1026R	2	13.50															O	P	Q	R					
MSU Line F1029	2	13.40															O	P	Q	R					
MSU Line F2039	2	12.900																P	Q	R					
MSU Line F1049	2	12.50																P	Q	R					
MSU Line F2020	2	12.00																	Q	R					
MSU Line F2038	2	11.950																	Q	R					
MSU Line F1032R	2	11.900																	Q	R					
MSU Line F2034	2	11.700																		R					

Means that do not share a letter are significantly different.

Figure 13.4. Raw Data for 2014 Total Ferulic Acid Content

One-way ANOVA: C2 versus C1

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	38	113361	2983	1.92	0.023
Error	39	60519	1552		
Total	77	173880			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
39.3924	65.20%	31.28%	0.00%

Means

C1	N	Mean	StDev	95% CI
Ambassador	2	304.72	2.87	(248.38, 361.07)
Aubrey	2	302.9	23.0	(246.6, 359.2)
F0013R	2	330.3	33.2	(274.0, 386.7)
F2010	2	351.0	20.7	(294.7, 407.4)
Jupiter	2	297.7	34.0	(241.3, 354.0)
MSU Line F1026R	2	370.29	7.27	(313.95, 426.63)
MSU Line F1027	2	352.693	1.145	(296.351, 409.034)
MSU Line F1029	2	399.3	49.4	(342.9, 455.6)
MSU Line F1047	2	350.7	60.8	(294.3, 407.0)
MSU Line F1048	2	388.02	9.70	(331.68, 444.36)
MSU Line F1049	2	355.637	1.296	(299.296, 411.979)
MSU Line F2002	2	342.8	65.0	(286.5, 399.2)
MSU Line F2003	2	414.5	45.5	(358.2, 470.9)
MSU Line F2005	2	303.672	0.496	(247.330, 360.013)
MSU Line F2008	2	372.0	16.4	(315.7, 428.4)
MSU Line F2009	2	386.6	19.0	(330.3, 443.0)
MSU Line F2012	2	414.857	0.582	(358.516, 471.198)
MSU Line F2014R	2	434.6	114.2	(378.2, 490.9)
MSU Line F2015	2	340.90	6.15	(284.55, 397.24)
MSU Line F2016	2	375.44	9.23	(319.10, 431.78)
MSU Line F2018	2	344.4	39.4	(288.1, 400.7)
MSU Line F2019	2	329.2	31.2	(272.8, 385.5)
MSU Line F2020	2	356.30	3.11	(299.96, 412.64)
MSU Line F2021	2	348.5	26.9	(292.2, 404.9)
MSU Line F2022	2	340.8	53.3	(284.5, 397.2)

Table 13.4 (cont'd)

```
MSU Line F2024R  2    363.3   26.0  (  306.9,   419.6)
MSU Line F2028R  2    442.2  100.9  (  385.9,   498.6)
MSU Line F2029R  2    429.2   28.7  (  372.9,   485.6)
MSU Line F2030   2    389.8   82.9  (  333.5,   446.2)
MSU Line F2031   2    362.9   23.0  (  306.6,   419.3)
MSU Line F2033   2   306.77   7.21  ( 250.43,  363.11)
MSU Line F2034   2    372.9   28.6  (  316.6,   429.3)
MSU Line F2037   2   353.15   2.52  ( 296.81,  409.49)
MSU Line F2038   2    399.1   14.3  (  342.7,   455.4)
MSU Line F2039   2    428.5   22.5  (  372.2,   484.9)
MSU Line F2042   2   325.24  10.72  ( 268.90,  381.58)
Unnamed 1        2    374.4   32.5  (  318.1,   430.7)
Unnamed 2        2   347.03   6.06  ( 290.69,  403.38)
Unnamed 3        2    406.5   54.1  (  350.2,   462.8)

Pooled StDev = 39.3924
```

Fisher Pairwise Comparisons

```
Grouping Information Using the Fisher LSD Method and 95% Confidence

C1               N     Mean  Grouping
MSU Line F2028R  2    442.2  A
MSU Line F2014R  2    434.6  A B
MSU Line F2029R  2    429.2  A B C
MSU Line F2039   2    428.5  A B C
MSU Line F2012   2  414.857  A B C D
MSU Line F2003   2    414.5  A B C D
Unnamed 3        2    406.5  A B C D E
MSU Line F1029   2    399.3  A B C D E F
MSU Line F2038   2    399.1  A B C D E F
MSU Line F2030   2    389.8  A B C D E F
MSU Line F1048   2   388.02  A B C D E F
MSU Line F2009   2    386.6  A B C D E F
MSU Line F2016   2   375.44  A B C D E F G
Unnamed 1        2    374.4  A B C D E F G
MSU Line F2034   2    372.9  A B C D E F G
MSU Line F2008   2    372.0  A B C D E F G
MSU Line F1026R  2   370.29  A B C D E F G
MSU Line F2024R  2    363.3  A B C D E F G
MSU Line F2031   2    362.9  A B C D E F G
MSU Line F2020   2   356.30    B C D E F G
MSU Line F1049   2  355.637    B C D E F G
MSU Line F2037   2   353.15      C D E F G
MSU Line F1027   2  352.693      C D E F G
F2010            2    351.0      C D E F G
MSU Line F1047   2    350.7      C D E F G
MSU Line F2021   2    348.5        D E F G
Unnamed 2        2   347.03        D E F G
MSU Line F2018   2    344.4        D E F G
MSU Line F2002   2    342.8        D E F G
```

Table 13.4 (cont'd)

```
MSU Line F2015   2   340.90        D E F G
```

MSU Line F2022	2	340.8	D E F G
F0013R	2	330.3	E F G
MSU Line F2019	2	329.2	E F G
MSU Line F2042	2	325.24	F G
MSU Line F2033	2	306.77	G
Ambassador	2	304.72	G
MSU Line F2005	2	303.672	G
Aubrey	2	302.9	G
Jupiter	2	297.7	G

Means that do not share a letter are significantly different.

Table 13.5. Raw Data for 2014 Bound Ferulic Acid Content

One-way ANOVA: C2 versus C1

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	38	110382	2905	2.05	0.014
Error	39	55308	1418		
Total	77	165690			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
37.6584	66.62%	34.10%	0.00%

Means

C1	N	Mean	StDev	95% CI
Ambassador	2	323.536	0.863	(269.675, 377.398)
Aubrey	2	333.81	6.01	(279.95, 387.67)
F0013R	2	368.2	37.8	(314.3, 422.0)
F2010	2	371.01	8.69	(317.15, 424.87)
Jupiter	2	316.3	36.3	(262.4, 370.1)
MSU Line F1026R	2	388.48	3.85	(334.62, 442.34)
MSU Line F1027	2	375.06	12.52	(321.20, 428.93)
MSU Line F1029	2	418.5	48.5	(364.7, 472.4)
MSU Line F1047	2	386.6	68.7	(332.7, 440.5)
MSU Line F1048	2	403.12	9.52	(349.26, 456.98)
MSU Line F1049	2	373.20	2.09	(319.34, 427.06)
MSU Line F2002	2	367.9	49.6	(314.1, 421.8)
MSU Line F2003	2	432.0	45.2	(378.1, 485.8)
MSU Line F2005	2	317.748	0.742	(263.886, 371.609)
MSU Line F2008	2	390.3	14.9	(336.4, 444.2)
MSU Line F2009	2	402.7	19.5	(348.9, 456.6)
MSU Line F2012	2	429.427	0.105	(375.566, 483.288)
MSU Line F2014R	2	457.5	113.7	(403.6, 511.4)
MSU Line F2015	2	356.32	7.31	(302.46, 410.18)
MSU Line F2016	2	396.15	9.65	(342.29, 450.01)
MSU Line F2018	2	367.6	35.9	(313.8, 421.5)
MSU Line F2019	2	353.4	30.1	(299.6, 407.3)
MSU Line F2020	2	372.68	1.74	(318.82, 426.55)
MSU Line F2021	2	381.4	20.8	(327.6, 435.3)

Table 13.5 (cont'd)

MSU Line F2022	2	360.3	51.1	(306.4,	414.1)
MSU Line F2024R	2	381.5	27.3	(327.7,	435.4)
MSU Line F2028R	2	467.2	93.6	(413.4,	521.1)
MSU Line F2029R	2	451.7	26.2	(397.8,	505.6)
MSU Line F2030	2	421.1	68.0	(367.2,	474.9)
MSU Line F2031	2	384.9	19.9	(331.0,	438.7)
MSU Line F2033	2	338.21	11.21	(284.35,	392.07)
MSU Line F2034	2	396.9	20.1	(343.1,	450.8)
MSU Line F2037	2	370.44	2.64	(316.58,	424.30)
MSU Line F2038	2	413.35	9.07	(359.49,	467.21)
MSU Line F2039	2	456.5	41.2	(402.7,	510.4)
MSU Line F2042	2	346.3	14.7	(292.4,	400.2)
Unnamed 1	2	391.3	35.7	(337.4,	445.1)
Unnamed 2	2	365.751	0.443	(311.890,	419.612)
Unnamed 3	2	422.4	51.8	(368.6,	476.3)

Pooled StDev = 37.6584

Fisher Pairwise Comparisons

Grouping Information Using the Fisher LSD Method and 95% Confidence

C1	N	Mean	Grouping
MSU Line F2028R	2	467.2	A
MSU Line F2014R	2	457.5	A B
MSU Line F2039	2	456.5	A B
MSU Line F2029R	2	451.7	A B
MSU Line F2003	2	432.0	A B C
MSU Line F2012	2	429.427	A B C D
Unnamed 3	2	422.4	A B C D E
MSU Line F2030	2	421.1	A B C D E
MSU Line F1029	2	418.5	A B C D E
MSU Line F2038	2	413.35	A B C D E F
MSU Line F1048	2	403.12	A B C D E F G
MSU Line F2009	2	402.7	A B C D E F G
MSU Line F2034	2	396.9	A B C D E F G H
MSU Line F2016	2	396.15	A B C D E F G H
Unnamed 1	2	391.3	A B C D E F G H I
MSU Line F2008	2	390.3	B C D E F G H I
MSU Line F1026R	2	388.48	B C D E F G H I
MSU Line F1047	2	386.6	B C D E F G H I
MSU Line F2031	2	384.9	B C D E F G H I
MSU Line F2024R	2	381.5	B C D E F G H I
MSU Line F2021	2	381.4	B C D E F G H I
MSU Line F1027	2	375.06	C D E F G H I
MSU Line F1049	2	373.20	C D E F G H I
MSU Line F2020	2	372.68	C D E F G H I
F2010	2	371.01	C D E F G H I
MSU Line F2037	2	370.44	C D E F G H I
F0013R	2	368.2	C D E F G H I
MSU Line F2002	2	367.9	C D E F G H I

Table 13.5 (cont'd)

MSU Line F2018	2	367.6	C D E F G H I

Unnamed 2	2	365.751	C D E F G H I
MSU Line F2022	2	360.3	C D E F G H I
MSU Line F2015	2	356.32	C D E F G H I
MSU Line F2019	2	353.4	D E F G H I
MSU Line F2042	2	346.3	E F G H I
MSU Line F2033	2	338.21	F G H I
Aubrey	2	333.81	G H I
Ambassador	2	323.536	H I
MSU Line F2005	2	317.748	I
Jupiter	2	316.3	I

Means that do not share a letter are significantly different.

Table 13.6. Raw Data for 2014 Free Ferulic Acid Content

One-way ANOVA: C2 versus C1

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	38	2867	75.46	1.62	0.069
Error	39	1816	46.57		
Total	77	4684			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6.82416	61.22%	23.44%	0.00%

Means

C1	N	Mean	StDev	95% CI
Ambassador	2	18.81	2.01	(9.05, 28.57)
Aubrey	2	30.9	17.0	(21.1, 40.7)
F0013R	2	37.82	4.65	(28.06, 47.58)
F2010	2	19.98	12.04	(10.22, 29.74)
Jupiter	2	18.60	2.31	(8.84, 28.36)
MSU Line F1026R	2	18.19	3.42	(8.43, 27.95)
MSU Line F1027	2	22.37	11.37	(12.61, 32.13)
MSU Line F1029	2	19.255	0.844	(9.494, 29.015)
MSU Line F1047	2	35.95	7.83	(26.19, 45.71)
MSU Line F1048	2	15.096	0.181	(5.336, 24.856)
MSU Line F1049	2	17.563	0.796	(7.803, 27.324)
MSU Line F2002	2	25.1	15.4	(15.3, 34.9)
MSU Line F2003	2	17.473	0.288	(7.713, 27.233)
MSU Line F2005	2	14.076	1.238	(4.316, 23.836)
MSU Line F2008	2	18.27	1.58	(8.51, 28.04)
MSU Line F2009	2	16.083	0.516	(6.322, 25.843)
MSU Line F2012	2	14.570	0.687	(4.810, 24.330)
MSU Line F2014R	2	22.930	0.526	(13.169, 32.690)
MSU Line F2015	2	15.426	1.162	(5.666, 25.186)
MSU Line F2016	2	20.709	0.419	(10.948, 30.469)
MSU Line F2018	2	23.23	3.51	(13.47, 32.99)
MSU Line F2019	2	24.231	1.099	(14.471, 33.991)
MSU Line F2020	2	16.38	4.84	(6.62, 26.14)
MSU Line F2021	2	32.90	6.19	(23.14, 42.66)
MSU Line F2022	2	19.44	2.21	(9.68, 29.20)
MSU Line F2024R	2	18.244	1.289	(8.484, 28.005)

Table 13.6 (cont'd)

```
MSU Line F2028R  2    24.99    7.34  ( 15.23,   34.75)
MSU Line F2029R  2    22.47    2.54  ( 12.71,   32.23)
MSU Line F2030   2     31.2    15.0  (  21.5,    41.0)
MSU Line F2031   2    21.93    3.11  ( 12.17,   31.69)
MSU Line F2033   2    31.44    4.00  ( 21.68,   41.20)
MSU Line F2034   2    24.00    8.55  ( 14.24,   33.76)
MSU Line F2037   2  17.2940  0.1247  (7.5336, 27.0543)
MSU Line F2038   2    14.27    5.20  (  4.51,   24.03)
MSU Line F2039   2     28.0    18.7  (  18.2,    37.8)
MSU Line F2042   2    21.06    3.96  ( 11.29,   30.82)
Unnamed 1        2    16.89    3.22  (  7.13,   26.65)
Unnamed 2        2    18.72    5.62  (  8.96,   28.48)
Unnamed 3        2    15.93    2.21  (  6.17,   25.69)

Pooled StDev = 6.82416
```

Fisher Pairwise Comparisons

```
Grouping Information Using the Fisher LSD Method and 95% Confidence

C1               N     Mean  Grouping
F0013R           2    37.82  A
MSU Line F1047   2    35.95  A B
MSU Line F2021   2    32.90  A B C
MSU Line F2033   2    31.44  A B C D
MSU Line F2030   2     31.2  A B C D E
Aubrey           2     30.9  A B C D E F
MSU Line F2039   2     28.0  A B C D E F G
MSU Line F2002   2     25.1  A B C D E F G H
MSU Line F2028R  2    24.99  A B C D E F G H
MSU Line F2019   2   24.231  A B C D E F G H
MSU Line F2034   2    24.00    B C D E F G H
MSU Line F2018   2    23.23    B C D E F G H
MSU Line F2014R  2   22.930    B C D E F G H
MSU Line F2029R  2    22.47    B C D E F G H
MSU Line F1027   2    22.37    B C D E F G H
MSU Line F2031   2    21.93      C D E F G H
MSU Line F2042   2    21.06      C D E F G H
MSU Line F2016   2   20.709      C D E F G H
F2010            2    19.98      C D E F G H
MSU Line F2022   2    19.44      C D E F G H
MSU Line F1029   2   19.255      C D E F G H
Ambassador       2    18.81        D E F G H
Unnamed 2        2    18.72        D E F G H
Jupiter          2    18.60        D E F G H
MSU Line F2008   2    18.27        D E F G H
MSU Line F2024R  2   18.244        D E F G H
MSU Line F1026R  2    18.19        D E F G H
MSU Line F1049   2   17.563          E F G H
MSU Line F2003   2   17.473          E F G H
MSU Line F2037   2  17.2940            F G H
```

Table 13.6 (cont'd)

```
Unnamed 1        2    16.89              G H
```

MSU Line F2020	2	16.38	G H
MSU Line F2009	2	16.083	G H
Unnamed 3	2	15.93	G H
MSU Line F2015	2	15.426	G H
MSU Line F1048	2	15.096	G H
MSU Line F2012	2	14.570	G H
MSU Line F2038	2	14.27	G H
MSU Line F2005	2	14.076	H

Means that do not share a letter are significantly different.

Table 13.7. Raw Data for 2013 Total Phenolic Acid Content

One-way ANOVA: C2 versus C1

```
* ERROR * Cannot draw the interval plot. Interval plots are illegible with
more than 45
          intervals.
* NOTE * Cannot draw the interval plot for the Fisher procedure. Interval
plots for
         comparisons are illegible with more than 45 intervals.

Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.

Analysis of Variance

Source   DF  Adj SS   Adj MS  F-Value  P-Value
C1       56  90.240  1.61142    42.34    0.000
Error   114   4.338  0.03806
Total   170  94.578

Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.195078  95.41%     93.16%      89.68%

Means

C1      N    Mean   StDev       95% CI
E0028   3   4.700   0.265  ( 4.477,  4.923)
E1007R  3   4.400   0.173  ( 4.177,  4.623)
E5011   3  7.0667  0.1155  (6.8436, 7.2898)
F1003R  3   4.000   0.000  ( 3.777,  4.223)
F1012   3   4.800   0.436  ( 4.577,  5.023)
F1014   3   6.800   0.173  ( 6.577,  7.023)
F1026R  3   4.567   0.208  ( 4.344,  4.790)
F1027   3  4.1000  0.1000  (3.8769, 4.3231)
F1029   3   5.567   0.231  ( 5.344,  5.790)
F1032R  3   4.900   0.000  ( 4.677,  5.123)
F1047   3  3.8500  0.0500  (3.6269, 4.0731)
F1048   3   4.750   0.250  ( 4.527,  4.973)
F1049   3   4.000   0.265  ( 3.777,  4.223)
F1050   3  4.5500  0.0500  (4.3269, 4.7731)
F1051   3  3.5500  0.0500  (3.3269, 3.7731)
F2001R  3  5.1500  0.0500  (4.9269, 5.3731)
F2002   3  4.4500  0.0500  (4.2269, 4.6731)
F2003   3   5.967   0.208  ( 5.744,  6.190)
F2004   3   4.667   0.231  ( 4.444,  4.890)
F2005   3  4.2500  0.0500  (4.0269, 4.4731)
F2006   3  5.2000  0.1000  (4.9769, 5.4231)
```

Table 13.7 (cont'd)

```
F2008   3   4.800   0.265  ( 4.577,  5.023)
F2009   3   4.600   0.361  ( 4.377,  4.823)
F2011   3   5.300   0.361  ( 5.077,  5.523)
F2012   3   4.000   0.173  ( 3.777,  4.223)
F2014R  3   5.000   0.173  ( 4.777,  5.223)
F2015   3  4.5500  0.1500  (4.3269, 4.7731)
F2016   3  4.3500  0.0500  (4.1269, 4.5731)
F2018   3   4.200   0.300  ( 3.977,  4.423)
F2019   3  3.9500  0.1500  (3.7269, 4.1731)
F2020   3  3.8500  0.1500  (3.6269, 4.0731)
F2021   3   5.200   0.529  ( 4.977,  5.423)
F2022   3   5.100   0.000  ( 4.877,  5.323)
F2024R  3  4.4500  0.0500  (4.2269, 4.6731)
F2025R  3  6.1000  0.1000  (5.8769, 6.3231)
F2027R  3   5.167   0.306  ( 4.944,  5.390)
F2028R  3  4.3500  0.1500  (4.1269, 4.5731)
F2029R  3   5.600   0.173  ( 5.377,  5.823)
F2030   3  6.3000  0.1000  (6.0769, 6.5231)
F2031   3   4.867   0.321  ( 4.644,  5.090)
F2032   3  3.8500  0.1500  (3.6269, 4.0731)
F2033   3   4.700   0.265  ( 4.477,  4.923)
F2034   3  4.3500  0.0500  (4.1269, 4.5731)
F2035   3  5.1500  0.0500  (4.9269, 5.3731)
F2036   3  4.2500  0.1500  (4.0269, 4.4731)
F2037   3   3.667   0.321  ( 3.444,  3.890)
F2038   3   5.000   0.200  ( 4.777,  5.223)
F2039   3  5.3500  0.1500  (5.1269, 5.5731)
F2040   3  5.0000  0.1000  (4.7769, 5.2231)
F2041   3  4.3500  0.0500  (4.1269, 4.5731)
F2042   3  4.0500  0.1500  (3.8269, 4.2731)
I5440   3  4.8500  0.1500  (4.6269, 5.0731)
I7067   3  3.9500  0.0500  (3.7269, 4.1731)
I7127   3  5.1500  0.1500  (4.9269, 5.3731)
I7826   3  4.9500  0.0500  (4.7269, 5.1731)
I9339   3  4.1500  0.0500  (3.9269, 4.3731)
I9340   3   4.400   0.000  ( 4.177,  4.623)

Pooled StDev = 0.195078
```

Fisher Pairwise Comparisons

```
Grouping Information Using the Fisher LSD Method and 95% Confidence

C1      N    Mean  Grouping
E5011   3  7.0667  A
F1014   3   6.800  A
F2030   3  6.3000    B
F2025R  3  6.1000    B C
F2003   3   5.967      C
F2029R  3   5.600        D
F1029   3   5.567        D
```

Table 13.7 (cont'd)

```
F2039   3  5.3500        D E
F2011   3   5.300        D E F
F2021   3   5.200          E F G
F2006   3  5.2000          E F G
F2027R  3   5.167          E F G H
I7127   3  5.1500          E F G H I
F2035   3  5.1500          E F G H I
F2001R  3  5.1500          E F G H I
F2022   3   5.100          E F G H I J
F2040   3  5.0000            F G H I J K
F2038   3   5.000            F G H I J K
F2014R  3   5.000            F G H I J K
I7826   3  4.9500              G H I J K L
F1032R  3   4.900              G H I J K L M
F2031   3   4.867                H I J K L M N
I5440   3  4.8500                  I J K L M N O
F2008   3   4.800                    J K L M N O
F1012   3   4.800                    J K L M N O
F1048   3   4.750                      K L M N O P
F2033   3   4.700                      K L M N O P Q
E0028   3   4.700                      K L M N O P Q
F2004   3   4.667                        L M N O P Q
F2009   3   4.600                          M N O P Q R
F1026R  3   4.567                            N O P Q R
F2015   3  4.5500                              O P Q R S
F1050   3  4.5500                              O P Q R S
F2024R  3  4.4500                                P Q R S T
F2002   3  4.4500                                P Q R S T
I9340   3   4.400                                  Q R S T U
E1007R  3   4.400                                  Q R S T U
F2034   3  4.3500                                    R S T U V
F2041   3  4.3500                                    R S T U V
F2016   3  4.3500                                    R S T U V
F2028R  3  4.3500                                    R S T U V
F2036   3  4.2500                                      S T U V W
F2005   3  4.2500                                      S T U V W
F2018   3   4.200                                        T U V W
I9339   3  4.1500                                        T U V W X
F1027   3  4.1000                                          U V W X
F2042   3  4.0500                                            V W X
F2012   3   4.000                                              W X
F1049   3   4.000                                              W X
F1003R  3   4.000                                              W X
I7067   3  3.9500                                              W X Y
F2019   3  3.9500                                              W X Y
F2032   3  3.8500                                                X Y Z
F2020   3  3.8500                                                X Y Z
F1047   3  3.8500                                                X Y Z
F2037   3   3.667                                                  Y Z
F1051   3  3.5500                                                    Z

Means that do not share a letter are significantly different.
```

Table 13.8. Raw Data for 2013 Bound Phenolic Content

One-way ANOVA: C2 versus C1

```
* ERROR * Cannot draw the interval plot. Interval plots are illegible with
more than 45
          intervals.
* NOTE * Cannot draw the interval plot for the Fisher procedure. Interval
plots for
         comparisons are illegible with more than 45 intervals.

Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.

Analysis of Variance

Source   DF  Adj SS   Adj MS  F-Value  P-Value
C1       56   6.517  0.11637     2.54    0.000
Error   114   5.213  0.04573
Total   170  11.730

Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.213838  55.56%     33.73%       0.01%


Means

C1      N    Mean   StDev       95% CI
E0028   3  2.1138  0.0873  (1.8692, 2.3584)
E1007R  3  1.9314  0.1413  (1.6868, 2.1759)
E5011   3   1.846   0.174  ( 1.601,  2.090)
F1003R  3  2.1276  0.1727  (1.8830, 2.3722)
F1012   3   2.340   0.346  ( 2.095,  2.584)
F1014   3   2.041   0.270  ( 1.796,  2.285)
F1026R  3   2.121   0.190  ( 1.877,  2.366)
F1027   3  2.2723  0.0714  (2.0277, 2.5168)
F1029   3  2.1279  0.0282  (1.8834, 2.3725)
F1032R  3  2.0670  0.0262  (1.8225, 2.3116)
F1047   3   2.093   0.360  ( 1.849,  2.338)
F1048   3   2.328   0.436  ( 2.083,  2.572)
F1049   3   2.450   0.324  ( 2.205,  2.694)
F1050   3  1.8887  0.0331  (1.6441, 2.1332)
F1051   3  1.9179  0.1531  (1.6733, 2.1625)
F2001R  3  2.0597  0.0491  (1.8151, 2.3043)
F2002   3  2.3967  0.1263  (2.1521, 2.6412)
F2003   3  1.9778  0.1562  (1.7332, 2.2224)
F2004   3  2.0627  0.1279  (1.8181, 2.3073)
F2005   3  2.1266  0.0791  (1.8820, 2.3711)
```

Table 13.8 (cont'd)

```
F2006   3  2.2269  0.1537  (1.9823, 2.4715)
F2008   3  2.1221  0.1578  (1.8775, 2.3666)
```

```
F2009   3   2.004   0.270  ( 1.760,  2.249)
F2011   3   2.282   0.245  ( 2.037,  2.526)
F2012   3   2.075   0.210  ( 1.830,  2.319)
F2014R  3  1.9889  0.1208  (1.7443, 2.2335)
F2015   3  2.0398  0.1520  (1.7952, 2.2844)
F2016   3   2.248   0.332  ( 2.003,  2.492)
F2018   3   2.552   0.416  ( 2.307,  2.796)
F2019   3   2.490   0.385  ( 2.246,  2.735)
F2020   3   2.346   0.220  ( 2.101,  2.590)
F2021   3   1.622   0.197  ( 1.378,  1.867)
F2022   3   2.209   0.236  ( 1.964,  2.453)
F2024R  3   2.223   0.287  ( 1.978,  2.467)
F2025R  3  2.3432  0.0578  (2.0987, 2.5878)
F2027R  3   1.918   0.186  ( 1.673,  2.163)
F2028R  3   2.170   0.288  ( 1.925,  2.415)
F2029R  3  2.2650  0.0621  (2.0204, 2.5095)
F2030   3  2.3733  0.0585  (2.1287, 2.6179)
F2031   3   2.108   0.175  ( 1.864,  2.353)
F2032   3  2.1560  0.1358  (1.9114, 2.4006)
F2033   3   1.699   0.173  ( 1.454,  1.943)
F2034   3  1.9805  0.1499  (1.7359, 2.2250)
F2035   3  1.7422  0.0863  (1.4977, 1.9868)
F2036   3  2.2444  0.1423  (1.9999, 2.4890)
F2037   3  2.3199  0.1639  (2.0754, 2.5645)
F2038   3  1.7902  0.0784  (1.5457, 2.0348)
F2039   3  2.2623  0.1069  (2.0177, 2.5069)
F2040   3  1.9902  0.1530  (1.7456, 2.2348)
F2041   3  2.1082  0.1335  (1.8636, 2.3528)
F2042   3  1.8751  0.1026  (1.6305, 2.1196)
I5440   3   2.309   0.665  ( 2.065,  2.554)
I7067   3  2.1964  0.1014  (1.9518, 2.4409)
I7127   3  2.2146  0.0877  (1.9700, 2.4592)
I7826   3   2.263   0.176  ( 2.018,  2.508)
I9339   3  2.0485  0.0332  (1.8039, 2.2931)
I9340   3  2.3329  0.0452  (2.0884, 2.5775)

Pooled StDev = 0.213838
```

Fisher Pairwise Comparisons

```
Grouping Information Using the Fisher LSD Method and 95% Confidence

C1      N    Mean  Grouping
F2018   3   2.552  A
F2019   3   2.490  A B
F1049   3   2.450  A B C
F2002   3  2.3967  A B C D
F2030   3  2.3733  A B C D E
F2020   3   2.346  A B C D E F
F2025R  3  2.3432  A B C D E F
```

Table 13.8 (cont'd)

```
F1012   3   2.340  A B C D E F
I9340   3  2.3329  A B C D E F G
F1048   3   2.328  A B C D E F G
```

```
F2037   3  2.3199  A B C D E F G H
I5440   3   2.309  A B C D E F G H
F2011   3   2.282  A B C D E F G H
F1027   3  2.2723  A B C D E F G H I
F2029R  3  2.2650  A B C D E F G H I
I7826   3   2.263  A B C D E F G H I J
F2039   3  2.2623  A B C D E F G H I J
F2016   3   2.248  A B C D E F G H I J
F2036   3  2.2444  A B C D E F G H I J
F2006   3  2.2269  A B C D E F G H I J K
F2024R  3   2.223  A B C D E F G H I J K
I7127   3  2.2146  A B C D E F G H I J K L
F2022   3   2.209  A B C D E F G H I J K L
I7067   3  2.1964    B C D E F G H I J K L
F2028R  3   2.170    B C D E F G H I J K L M
F2032   3  2.1560    B C D E F G H I J K L M
F1029   3  2.1279      C D E F G H I J K L M N
F1003R  3  2.1276      C D E F G H I J K L M N
F2005   3  2.1266      C D E F G H I J K L M N
F2008   3  2.1221      C D E F G H I J K L M N
F1026R  3   2.121      C D E F G H I J K L M N
E0028   3  2.1138      C D E F G H I J K L M N
F2031   3   2.108      C D E F G H I J K L M N
F2041   3  2.1082      C D E F G H I J K L M N
F1047   3   2.093        D E F G H I J K L M N
F2012   3   2.075        D E F G H I J K L M N O
F1032R  3  2.0670        D E F G H I J K L M N O
F2004   3  2.0627        D E F G H I J K L M N O
F2001R  3  2.0597        D E F G H I J K L M N O
I9339   3  2.0485          E F G H I J K L M N O
F1014   3   2.041          E F G H I J K L M N O P
F2015   3  2.0398          E F G H I J K L M N O P
F2009   3   2.004            F G H I J K L M N O P
F2040   3  1.9902              G H I J K L M N O P
F2014R  3  1.9889              G H I J K L M N O P
F2034   3  1.9805                H I J K L M N O P
F2003   3  1.9778                H I J K L M N O P
E1007R  3  1.9314                  I J K L M N O P Q
F2027R  3   1.918                    J K L M N O P Q
F1051   3  1.9179                    J K L M N O P Q
F1050   3  1.8887                      K L M N O P Q
F2042   3  1.8751                        L M N O P Q
E5011   3   1.846                          M N O P Q
F2038   3  1.7902                            N O P Q
F2035   3  1.7422                              O P Q
F2033   3   1.699                                P Q
F2021   3   1.622                                  Q
```

Means that do not share a letter are significantly different.

Table 13.9. Raw Data for 2013 Free Phenolic Content

One-way ANOVA: C2 versus C1

```
* ERROR * Cannot draw the interval plot. Interval plots are illegible with
more than 45
          intervals.
* NOTE * Cannot draw the interval plot for the Fisher procedure. Interval
plots for
         comparisons are illegible with more than 45 intervals.

Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.

Analysis of Variance

Source   DF  Adj SS  Adj MS  F-Value  P-Value
C1       56   97.58   1.743     1.11    0.319
Error   114  179.33   1.573
Total   170  276.91


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
1.25423  35.24%      3.43%       0.00%


Means

C1      N   Mean  StDev      95% CI
E0028   3  3.270  1.078  (1.836, 4.705)
E1007R  3  3.007  1.033  (1.573, 4.442)
E5011   3  5.848  1.034  (4.413, 7.282)
F1003R  3  2.516  1.286  (1.081, 3.950)
F1012   3  3.354  1.703  (1.919, 4.788)
F1014   3  5.436  1.009  (4.002, 6.871)
F1026R  3  3.266  1.328  (1.832, 4.701)
F1027   3  2.621  1.367  (1.187, 4.056)
F1029   3  4.100  1.082  (2.666, 5.534)
F1032R  3  3.513  1.201  (2.079, 4.948)
F1047   3  2.569  1.170  (1.135, 4.004)
F1048   3  3.122  1.631  (1.687, 4.556)
F1049   3  2.176  1.321  (0.741, 3.610)
F1050   3  3.297  1.129  (1.862, 4.731)
F1051   3  2.333  1.099  (0.898, 3.767)
F2001R  3  3.765  1.160  (2.330, 5.199)
F2002   3  2.822  1.453  (1.387, 4.256)
F2003   3  4.636  1.010  (3.201, 6.070)
F2004   3  3.340  1.265  (1.905, 4.774)
```

Table 13.9 (cont'd)

```
F2005   3  2.819  1.284  (1.384, 4.253)
F2006   3  3.737  1.190  (2.303, 5.172)
F2008   3  3.400  1.389  (1.966, 4.835)
F2009   3  3.128  0.933  (1.693, 4.562)
F2011   3  3.684  1.071  (2.249, 5.118)
F2012   3  2.481  1.143  (1.046, 3.915)
F2014R  3  3.584  1.053  (2.150, 5.019)
F2015   3  3.265  1.243  (1.831, 4.700)
F2016   3  2.741  1.439  (1.307, 4.176)
F2018   3   2.47   1.75  ( 1.04,  3.91)
F2019   3  2.400  1.513  (0.966, 3.834)
F2020   3  2.291  1.481  (0.856, 3.725)
F2021   3  3.874  0.701  (2.440, 5.309)
F2022   3  3.652  1.275  (2.217, 5.086)
F2024R  3  2.887  1.400  (1.452, 4.321)
F2025R  3  4.561  1.420  (3.126, 5.995)
F2027R  3  3.888  0.907  (2.454, 5.323)
F2028R  3  2.910  1.382  (1.476, 4.345)
F2029R  3  4.105  1.383  (2.670, 5.539)
F2030   3  4.737  1.441  (3.302, 6.171)
F2031   3  3.362  1.028  (1.927, 4.796)
F2032   3  2.421  1.369  (0.986, 3.855)
F2033   3  3.466  0.814  (2.032, 4.901)
F2034   3  3.071  1.066  (1.636, 4.505)
F2035   3  3.950  0.996  (2.516, 5.385)
F2036   3  2.793  1.392  (1.359, 4.228)
F2037   3  2.273  1.339  (0.839, 3.708)
F2038   3  3.757  0.913  (2.323, 5.192)
F2039   3  3.852  1.438  (2.417, 5.286)
F2040   3  3.648  1.260  (2.214, 5.083)
F2041   3  2.912  1.292  (1.478, 4.347)
F2042   3  2.785  0.971  (1.350, 4.219)
I5440   3  3.584  1.239  (2.149, 5.018)
I7067   3  2.499  1.308  (1.065, 3.934)
I7127   3  3.712  1.377  (2.277, 5.146)
I7826   3  3.372  1.324  (1.938, 4.807)
I9339   3  2.790  1.222  (1.355, 4.224)
I9340   3  2.830  1.360  (1.395, 4.264)

Pooled StDev = 1.25423
```

Fisher Pairwise Comparisons

```
Grouping Information Using the Fisher LSD Method and 95% Confidence

C1      N   Mean  Grouping
E5011   3  5.848  A
F1014   3  5.436  A B
F2030   3  4.737  A B C
F2003   3  4.636  A B C D
F2025R  3  4.561  A B C D E
F2029R  3  4.105  A B C D E F
```

Table 13.9 (cont'd)

F1029	3	4.100	A B C D E F
F2035	3	3.950	A B C D E F
F2027R	3	3.888	A B C D E F
F2021	3	3.874	A B C D E F
F2039	3	3.852	A B C D E F
F2001R	3	3.765	B C D E F
F2038	3	3.757	B C D E F
F2006	3	3.737	B C D E F
I7127	3	3.712	B C D E F
F2011	3	3.684	B C D E F
F2022	3	3.652	B C D E F
F2040	3	3.648	B C D E F
F2014R	3	3.584	B C D E F
I5440	3	3.584	B C D E F
F1032R	3	3.513	B C D E F
F2033	3	3.466	B C D E F
F2008	3	3.400	C D E F
I7826	3	3.372	C D E F
F2031	3	3.362	C D E F
F1012	3	3.354	C D E F
F2004	3	3.340	C D E F
F1050	3	3.297	C D E F
E0028	3	3.270	C D E F
F1026R	3	3.266	C D E F
F2015	3	3.265	C D E F
F2009	3	3.128	C D E F
F1048	3	3.122	C D E F
F2034	3	3.071	C D E F
E1007R	3	3.007	C D E F
F2041	3	2.912	C D E F
F2028R	3	2.910	C D E F
F2024R	3	2.887	C D E F
I9340	3	2.830	C D E F
F2002	3	2.822	C D E F
F2005	3	2.819	C D E F
F2036	3	2.793	C D E F
I9339	3	2.790	C D E F
F2042	3	2.785	C D E F
F2016	3	2.741	C D E F
F1027	3	2.621	D E F
F1047	3	2.569	E F
F1003R	3	2.516	F
I7067	3	2.499	F
F2012	3	2.481	F
F2018	3	2.47	F
F2032	3	2.421	F
F2019	3	2.400	F
F1051	3	2.333	F
F2020	3	2.291	F
F2037	3	2.273	F
F1049	3	2.176	F

Means that do not share a letter are significantly different.

Table 13.10. Raw Data for 2014 Total Phenolic Content

One-way ANOVA: C2 versus C1

* NOTE * Cannot draw the interval plot for the Fisher procedure. Interval plots for
comparisons are illegible with more than 45 intervals.

Method

Null hypothesis	All means are equal
Alternative hypothesis	At least one mean is different
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
C1	38	23.91	0.6292	2.77	0.000
Error	78	17.72	0.2271		
Total	116	41.63			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.476597	57.44%	36.70%	4.24%

Means

C1	N	Mean	StDev	95% CI
E0028	3	4.290	0.466	(3.743, 4.838)
E5011	3	5.297	0.631	(4.749, 5.845)
F0013R	3	4.505	0.356	(3.957, 5.053)
F1026R	3	5.728	0.561	(5.180, 6.276)
F1027	3	5.205	0.249	(4.658, 5.753)
F1029	3	5.098	0.565	(4.550, 5.646)
F1047	3	5.208	0.815	(4.660, 5.756)
F1048	3	4.903	0.874	(4.356, 5.451)
F1049	3	5.022	0.893	(4.474, 5.569)
F2002	3	5.786	0.630	(5.238, 6.334)
F2003	3	5.1284	0.1626	(4.5806, 5.6762)
F2005	3	4.7871	0.0466	(4.2393, 5.3349)
F2008	3	4.627	0.675	(4.079, 5.175)
F2009	3	5.4218	0.1161	(4.8740, 5.9696)
F2010	3	4.360	0.238	(3.812, 4.908)
F2012	3	4.4727	0.1164	(3.9249, 5.0205)
F2014R	3	5.173	0.233	(4.625, 5.721)
F2015	3	5.717	0.193	(5.170, 6.265)
F2016	3	5.479	0.624	(4.931, 6.027)
F2018	3	4.430	0.790	(3.882, 4.978)
F2019	3	5.141	0.256	(4.593, 5.689)
F2020	3	5.242	0.430	(4.695, 5.790)

Table 13.10 (cont'd)

F2021	3	5.1504	0.1158	(4.6026, 5.6982)
F2022	3	5.702	0.717	(5.154, 6.250)

```
F2024R  3   5.515   0.492  ( 4.967,  6.063)
F2028R  3   5.159   0.233  ( 4.611,  5.707)
F2029R  3   4.696   0.584  ( 4.148,  5.243)
F2030   3  5.0888  0.0748  (4.5410, 5.6366)
F2031   3  5.1282  0.0578  (4.5804, 5.6760)
F2033   3  5.2777  0.0531  (4.7299, 5.8255)
F2034   3   5.698   0.592  ( 5.150,  6.246)
F2037   3   3.996   0.223  ( 3.448,  4.544)
F2038   3   5.123   0.460  ( 4.575,  5.671)
F2039   3  5.0285  0.1370  (4.4807, 5.5763)
F2042   3   5.522   0.427  ( 4.974,  6.070)
I7826   3   5.681   0.351  ( 5.133,  6.229)
x1      3   5.976   0.185  ( 5.428,  6.524)
x2      3   5.250   0.841  ( 4.702,  5.798)
x3      3  4.9431  0.1466  (4.3953, 5.4909)

Pooled StDev = 0.476597
```

Fisher Pairwise Comparisons

```
Grouping Information Using the Fisher LSD Method and 95% Confidence

C1      N    Mean  Grouping
x1      3   5.976  A
F2002   3   5.786  A B
F1026R  3   5.728  A B
F2015   3   5.717  A B C
F2022   3   5.702  A B C
F2034   3   5.698  A B C
I7826   3   5.681  A B C
F2042   3   5.522  A B C D
F2024R  3   5.515  A B C D
F2016   3   5.479  A B C D
F2009   3  5.4218  A B C D E
E5011   3   5.297  A B C D E F
F2033   3  5.2777  A B C D E F G
x2      3   5.250  A B C D E F G
F2020   3   5.242  A B C D E F G H
F1047   3   5.208  A B C D E F G H
F1027   3   5.205  A B C D E F G H
F2014R  3   5.173    B C D E F G H I
F2028R  3   5.159    B C D E F G H I
F2021   3  5.1504    B C D E F G H I
F2019   3   5.141    B C D E F G H I
F2003   3  5.1284    B C D E F G H I J
F2031   3  5.1282    B C D E F G H I J
F2038   3   5.123    B C D E F G H I J
F1029   3   5.098    B C D E F G H I J
F2030   3  5.0888    B C D E F G H I J
F2039   3  5.0285    B C D E F G H I J K
```

Table 13.10 (cont'd)

```
F1049   3   5.022    B C D E F G H I J K
x3      3  4.9431      C D E F G H I J K
F1048   3   4.903        D E F G H I J K
```

```
F2005   3  4.7871        D E F G H I J K
F2029R  3   4.696          E F G H I J K L
F2008   3   4.627            F G H I J K L
F0013R  3   4.505              G H I J K L
F2012   3  4.4727               H I J K L
F2018   3   4.430                 I J K L
F2010   3   4.360                   J K L
E0028   3   4.290                     K L
F2037   3   3.996                       L
```

Means that do not share a letter are significantly different.

Table 13.11. Raw Data for 2014 Bound Phenolic Content

One-way ANOVA: C2 versus C1

```
* NOTE * Cannot draw the interval plot for the Fisher procedure. Interval
plots for
         comparisons are illegible with more than 45 intervals.


Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Analysis of Variance

Source   DF  Adj SS  Adj MS  F-Value  P-Value
C1       38   27.65  0.7277     1.89    0.009
Error    78   30.09  0.3858
Total   116   57.75

Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.621119  47.89%     22.50%       0.00%

Means

C1      N     Mean    StDev        95% CI
E0028   3    3.028    0.737  (  2.314,   3.742)
E5011   3    3.732    0.638  (  3.018,   4.446)
F0013R  3    3.771    0.310  (  3.057,   4.485)
F1026R  3   4.4918   0.1086  ( 3.7779,  5.2057)
F1027   3   3.7433   0.0733  ( 3.0294,  4.4572)
F1029   3    3.757    0.556  (  3.043,   4.471)
F1047   3    4.648    1.325  (  3.934,   5.362)
F1048   3    4.339    1.073  (  3.625,   5.053)
F1049   3    3.481    1.173  (  2.767,   4.195)
F2002   3    4.391    0.652  (  3.677,   5.105)
F2003   3    4.107    0.460  (  3.393,   4.821)
F2005   3   3.7699   0.1050  ( 3.0560,  4.4838)
F2008   3    3.613    1.013  (  2.899,   4.327)
F2009   3    4.304    0.375  (  3.590,   5.018)
F2010   3   2.8708   0.1362  ( 2.1568,  3.5847)
F2012   3    3.440    0.390  (  2.726,   4.154)
F2014R  3  3.82214  0.00245  (3.10821, 4.53606)
F2015   3   4.6072   0.1625  ( 3.8932,  5.3211)
F2016   3    3.945    0.757  (  3.231,   4.658)
F2018   3    3.767    1.062  (  3.053,   4.481)
F2019   3   3.5131   0.0777  ( 2.7992,  4.2270)
F2020   3    4.563    0.597  (  3.849,   5.277)
```

Table 13.11 (cont'd)

```
F2021   3    3.691    0.489  (  2.977,   4.405)
F2022   3    4.656    0.740  (  3.942,   5.369)
F2024R  3    4.424    0.813  (  3.710,   5.138)
F2028R  3    3.680    0.202  (  2.966,   4.394)
F2029R  3    3.106    0.739  (  2.393,   3.820)
F2030   3    3.965    0.407  (  3.252,   4.679)
F2031   3   3.8426   0.0590  ( 3.1287,  4.5565)
F2033   3    4.113    0.330  (  3.399,   4.827)
F2034   3    4.136    0.827  (  3.422,   4.850)
F2037   3  3.01863  0.00262  (2.30470, 3.73255)
F2038   3  3.53641  0.00875  (2.82248, 4.25033)
F2039   3    4.190    0.380  (  3.476,   4.904)
F2042   3    4.537    0.333  (  3.823,   5.251)
I7826   3    4.161    0.779  (  3.447,   4.875)
x1      3    4.911    0.343  (  4.197,   5.625)
x2      3    3.731    1.026  (  3.018,   4.445)
x3      3    3.968    0.536  (  3.254,   4.682)

Pooled StDev = 0.621119
```

Fisher Pairwise Comparisons

```
Grouping Information Using the Fisher LSD Method and 95% Confidence

C1      N     Mean  Grouping
x1      3    4.911  A
F2022   3    4.656  A B
F1047   3    4.648  A B
F2015   3   4.6072  A B C
F2020   3    4.563  A B C
F2042   3    4.537  A B C D
F1026R  3   4.4918  A B C D E
F2024R  3    4.424  A B C D E F
F2002   3    4.391  A B C D E F
F1048   3    4.339  A B C D E F
F2009   3    4.304  A B C D E F
F2039   3    4.190  A B C D E F
I7826   3    4.161  A B C D E F
F2034   3    4.136  A B C D E F
F2033   3    4.113  A B C D E F G
F2003   3    4.107  A B C D E F G
x3      3    3.968  A B C D E F G H
F2030   3    3.965  A B C D E F G H
F2016   3    3.945  A B C D E F G H
F2031   3   3.8426    B C D E F G H I
F2014R  3  3.82214    B C D E F G H I
F0013R  3    3.771    B C D E F G H I
F2005   3   3.7699    B C D E F G H I
F2018   3    3.767    B C D E F G H I
F1029   3    3.757    B C D E F G H I
F1027   3   3.7433    B C D E F G H I
E5011   3    3.732    B C D E F G H I
```

Table 13.11 (cont'd)

x2	3	3.731	B C D E F G H I
F2021	3	3.691	B C D E F G H I
F2028R	3	3.680	B C D E F G H I
F2008	3	3.613	C D E F G H I
F2038	3	3.53641	D E F G H I
F2019	3	3.5131	E F G H I
F1049	3	3.481	F G H I
F2012	3	3.440	F G H I
F2029R	3	3.106	G H I
E0028	3	3.028	H I
F2037	3	3.01863	H I
F2010	3	2.8708	I

Means that do not share a letter are significantly different.

Table 13.12. Raw Data for 2014 Free Phenolic Content

Interval Plot of C2 vs C1

One-way ANOVA: C2 versus C1

```
* NOTE * Cannot draw the interval plot for the Fisher procedure. Interval
plots for
         comparisons are illegible with more than 45 intervals.


Method

Null hypothesis         All means are equal
Alternative hypothesis  At least one mean is different
Significance level      α = 0.05

Equal variances were assumed for the analysis.


Analysis of Variance

Source   DF  Adj SS   Adj MS  F-Value  P-Value
C1       38   5.125  0.13486     2.24    0.001
Error    78   4.695  0.06020
Total   116   9.820


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.245349  52.19%     28.89%       0.00%


Means

C1      N     Mean    StDev        95% CI
E0028   3   1.4495   0.0292  ( 1.1675,  1.7315)
E5011   3   1.3476   0.0321  ( 1.0656,  1.6296)
F0013R  3   0.8457   0.0402  ( 0.5637,  1.1277)
F1026R  3    1.373    0.345  (  1.091,   1.655)
F1027   3    1.442    0.397  (  1.160,   1.724)
F1029   3   1.1249   0.0629  ( 0.8429,  1.4069)
F1047   3    1.061    0.439  (  0.779,   1.343)
F1048   3    0.980    0.247  (  0.698,   1.262)
F1049   3   1.1686   0.0193  ( 0.8866,  1.4506)
F2002   3   1.3404   0.0211  ( 1.0583,  1.6224)
F2003   3    1.186    0.335  (  0.904,   1.468)
F2005   3   1.0475   0.0443  ( 0.7655,  1.3295)
F2008   3    1.228    0.277  (  0.946,   1.510)
F2009   3    1.077    0.312  (  0.795,   1.359)
F2010   3   1.4497   0.1201  ( 1.1677,  1.7317)
F2012   3    1.079    0.323  (  0.797,   1.361)
```

Table 13.12 (cont'd)

```
F2014R  3    1.298    0.348  (  1.016,   1.580)
F2015   3   1.1298   0.0632  ( 0.8478,  1.4118)
F2016   3   1.1160   0.1362  ( 0.8340,  1.3980)
F2018   3  1.12308  0.00543  (0.84107, 1.40508)
F2019   3    1.650    0.323  (  1.368,   1.932)
F2020   3   0.9182   0.1028  ( 0.6362,  1.2002)
F2021   3    1.508    0.415  (  1.226,   1.790)
F2022   3   0.8330   0.0767  ( 0.5510,  1.1150)
F2024R  3    1.192    0.269  (  0.910,   1.474)
F2028R  3   1.3607   0.1160  ( 1.0787,  1.6427)
F2029R  3   1.2426   0.1031  ( 0.9606,  1.5246)
F2030   3    1.241    0.278  (  0.959,   1.523)
F2031   3   1.3360   0.0542  ( 1.0540,  1.6180)
F2033   3    1.254    0.218  (  0.972,   1.536)
F2034   3   1.3233   0.0501  ( 1.0413,  1.6053)
F2037   3    0.976    0.258  (  0.694,   1.258)
F2038   3    1.589    0.468  (  1.307,   1.871)
F2039   3   0.9818   0.1656  ( 0.6998,  1.2638)
F2042   3   0.9745   0.1558  ( 0.6925,  1.2565)
I7826   3    1.744    0.254  (  1.462,   2.026)
x1      3    1.188    0.511  (  0.906,   1.470)
x2      3   1.3340   0.0578  ( 1.0520,  1.6160)
x3      3    1.180    0.270  (  0.898,   1.462)

Pooled StDev = 0.245349
```

Fisher Pairwise Comparisons

```
Grouping Information Using the Fisher LSD Method and 95% Confidence

C1      N     Mean  Grouping
I7826   3    1.744  A
F2019   3    1.650  A B
F2038   3    1.589  A B C
F2021   3    1.508  A B C D
F2010   3   1.4497  A B C D E
E0028   3   1.4495  A B C D E
F1027   3    1.442  A B C D E F
F1026R  3    1.373  A B C D E F G
F2028R  3   1.3607  A B C D E F G
E5011   3   1.3476  A B C D E F G
F2002   3   1.3404    B C D E F G
F2031   3   1.3360    B C D E F G
x2      3   1.3340    B C D E F G
F2034   3   1.3233    B C D E F G
F2014R  3    1.298    B C D E F G H
F2033   3    1.254    B C D E F G H
F2029R  3   1.2426      C D E F G H I
F2030   3    1.241      C D E F G H I
F2008   3    1.228      C D E F G H I J
F2024R  3    1.192      C D E F G H I J
x1      3    1.188        D E F G H I J
```

Table 13.12 (cont'd)

F2003	3	1.186	D E F G H I J
x3	3	1.180	D E F G H I J
F1049	3	1.1686	D E F G H I J
F2015	3	1.1298	D E F G H I J
F1029	3	1.1249	D E F G H I J
F2018	3	1.12308	D E F G H I J
F2016	3	1.1160	D E F G H I J
F2012	3	1.079	E F G H I J
F2009	3	1.077	E F G H I J
F1047	3	1.061	E F G H I J
F2005	3	1.0475	F G H I J
F2039	3	0.9818	G H I J
F1048	3	0.980	G H I J
F2037	3	0.976	G H I J
F2042	3	0.9745	G H I J
F2020	3	0.9182	H I J
F0013R	3	0.8457	I J
F2022	3	0.8330	J

Means that do not share a letter are significantly different.

APPENDIX D. Colorimetric Analysis Raw Data

Table 14.1. Phenolic Acid Contents[1] of Selected 2013 MSU Wheat Breeding Varieties

Variety Number	Variety Name	Total Phenolic Content (mg GAE/g)	Bound Phenolic Content (mg GAE/g)	Free Phenolic Content (mg GAE/g)
1	F1014	4.2 ± 0.2	2.4 ± 0.4	2.2 ± 0.2
2	F1026R	5.7 ± 0.2	2.5 ± 0.0	2.0 ± 0.2
3	F1012	4.8 ± 0.4	2.6 ± 0.7	2.2 ± 0.3
4	F1003R	4.0 ± 0.0	1.9 ± 0.2	2.1 ± 0.2
5	F1029	3.8 ± 0.2	3.4 ± 0.2	2.1 ± 0.0
6	F1047	3.7 ± 0.1	1.8 ± 0.4	2.0 ± 0.4
7	F1032R	5.1 ± 0.0	2.8 ± 0.0	2.1 ± 0.0
8	F1027	4.4 ± 0.1	1.9 ± 0.1	2.2 ± 0.1
9	F1049	3.8 ± 0.3	1.5 ± 0.1	2.5 ± 0.4
10	F1051	3.8 ± 0.1	1.6 ± 0.1	1.9 ± 0.2
11	F1050	4.5 ± 0.0	2.7 ± 0.1	1.9 ± 0.0
12	F1048	4.9 ± 0.3	2.6 ± 0.8	2.1 ± 0.4
13	F2031	4.8 ± 0.3	2.7 ± 0.6	2.2 ± 0.2
14	F2033	4.6 ± 0.3	2.9 ± 0.3	1.8 ± 0.0
15	F2034	4.2 ± 0.1	2.3 ± 0.3	2.0 ± 0.2
16	F2032	3.9 ± 0.2	1.8 ± 0.3	2.1 ± 0.1
17	F2037	3.6 ± 0.3	1.4 ± 0.4	2.2 ± 0.1
18	F2035	4.8 ± 0.1	3.4 ± 0.0	1.8 ± 0.1
19	F2041	4.4 ± 0.1	2.3 ± 0.3	2.1 ± 0.2
20	F2036	4.3 ± 0.2	2.1 ± 0.1	2.2 ± 0.1
21	F2038	5.3 ± 0.2	3.2 ± 0.3	1.8 ± 0.0
22	F2040	4.9 ± 0.1	3.1 ± 0.3	1.9 ± 0.2
23	F2042	4.3 ± 0.2	2.1 ± 0.3	1.9 ± 0.1
24	F2039	5.4 ± 0.2	3.1 ± 0.4	2.2 ± 0.1
25	F2001R	5.0 ± 0.1	3.1 ± 0.1	2.0 ± 0.0
26	F2006	5.0 ± 0.1	3.0 ± 0.4	2.2 ± 0.2
27	F2005	5.1 ± 0.0	2.1 ± 0.2	2.1 ± 0.1
28	F2015	4.7 ± 0.2	2.6 ± 0.0	2.0 ± 0.2
29	F2009	4.9 ± 0.4	2.4 ± 0.3	2.2 ± 0.1
30	F2012	4.6 ± 0.2	1.9 ± 0.1	2.1 ± 0.2
31	F2008	4.3 ± 0.3	2.8 ± 0.4	2.0 ± 0.0
32	F2011	5.0 ± 0.4	2.9 ± 0.5	2.4 ± 0.1
33	F2014R	5.3 ± 0.2	2.9 ± 0.1	2.1 ± 0.1
34	F2003	6.0 ± 0.2	3.9 ± 0.3	2.1 ± 0.0
35	F2004	4.9 ± 0.2	2.6 ± 0.1	2.0 ± 0.1
36	F2002	4.8 ± 0.0	2.1 ± 0.2	2.3 ± 0.1
37	F2028R	4.3 ± 0.2	2.3 ± 0.2	2.0 ± 0.1
38	F2020	3.8 ± 0.2	1.6 ± 0.2	2.2 ± 0.0
39	F2024R	4.8 ± 0.0	2.4 ± 0.3	2.1 ± 0.2
40	F2018	4.2 ± 0.3	1.9 ± 0.6	2.3 ± 0.2
41	F2019	3.9 ± 0.2	1.7 ± 0.4	2.3 ± 0.2

Table 14.1 (cont'd)

42	**F2016**	**4.3**	**±**	**0.1**	**2.2**	**±**	**0.5**	**2.2**	**±**	**0.4**
43	F2022	5.3	±	0.0	3.0	±	0.2	2.1	±	0.2
44	F2025R	5.9	±	0.1	3.8	±	0.1	2.3	±	0.0
45	F2021	5.1	±	0.5	3.5	±	0.5	1.7	±	0.1
46	F2030	6.0	±	0.1	4.0	±	0.2	2.3	±	0.0
47	F2029R	5.6	±	0.2	3.4	±	0.2	2.2	±	0.0
48	F2027R	4.8	±	0.3	3.1	±	0.7	2.0	±	0.2
49	Aubrey	4.9	±	0.0	2.7	±	0.2	2.3	±	0.2
50	Hopewell	4.7	±	0.2	3.0	±	0.2	2.2	±	0.0
51	Ambassador	5.2	±	0.3	2.5	±	0.5	2.2	±	0.0
52	Jupiter	6.8	±	0.1	5.3	±	0.3	1.8	±	0.2
53	Red Ruby	3.9	±	0.2	2.4	±	0.0	2.0	±	0.1
54	VA09W-188WS	4.1	±	0.1	2.1	±	0.1	2.0	±	0.0
55	Cayuga	5.1	±	0.2	2.5	±	0.7	2.3	±	0.4
56	VA09W-192WS	4.2	±	0.0	2.1	±	0.1	2.3	±	0.1
57	Caledonia	4.1	±	0.1	1.7	±	0.2	2.2	±	0.1

[1] Results are expressed as milligrams of gallic acid equivalent per gram of sample, with the data presented as means ± standard deviation (n=3).

Table 14.2. Phenolic Acid Contents[1] of Selected 2014 MSU Wheat Breeding Varieties

Variety Number	Variety Name	Total Phenolic Content (mg GAE/g)	Bound Phenolic Content (mg GAE/g)	Free Phenolic Content (mg GAE/g)
2	F1026R	5.8 ± 0.5	4.4 ± 0.0	1.4 ± 0.6
5	F1029	5.1 ± 0.4	4.0 ± 0.5	1.1 ± 0.1
6	F1047	5.2 ± 0.5	4.3 ± 1.6	0.9 ± 0.5
8	F1027	5.2 ± 0.4	3.8 ± 0.1	1.4 ± 0.3
9	F1049	5.0 ± 0.3	3.8 ± 1.4	1.2 ± 0.0
12	F1048	4.9 ± 0.4	4.0 ± 1.2	1.0 ± 0.3
13	F2031	5.2 ± 0.0	3.8 ± 0.1	1.3 ± 0.1
14	F2033	5.2 ± 0.1	4.0 ± 0.4	1.2 ± 0.2
15	F2034	5.7 ± 0.5	4.4 ± 1.0	1.3 ± 0.1
17	F2037	4.0 ± 0.4	3.0 ± 0.0	1.0 ± 0.4
21	F2038	5.1 ± 0.7	3.5 ± 0.0	1.6 ± 0.7
23	F2042	5.5 ± 0.5	4.5 ± 0.5	1.0 ± 0.2
24	F2039	5.1 ± 0.3	4.1 ± 0.5	1.0 ± 0.2
27	F2005	4.8 ± 0.1	3.7 ± 0.1	1.0 ± 0.1
28	F2015	5.7 ± 0.3	4.6 ± 0.2	1.1 ± 0.1
29	F2009	5.5 ± 0.0	4.4 ± 0.5	1.1 ± 0.4
30	F2012	4.4 ± 0.0	3.3 ± 0.5	1.1 ± 0.5
31	F2008	4.5 ± 0.6	3.3 ± 1.2	1.2 ± 0.4
33	F2014R	5.1 ± 0.3	3.8 ± 0.0	1.3 ± 0.3
34	F2003	5.1 ± 0.2	3.9 ± 0.5	1.2 ± 0.3
36	F2002	5.9 ± 0.4	4.6 ± 0.8	1.3 ± 0.0
37	F2028R	5.0 ± 0.2	3.6 ± 0.2	1.4 ± 0.0
38	F2020	5.3 ± 0.6	4.4 ± 0.7	0.9 ± 0.1
39	F2024R	5.4 ± 0.6	4.2 ± 1.0	1.2 ± 0.4
40	F2018	4.7 ± 0.4	3.5 ± 1.4	1.1 ± 0.0
41	F2019	5.1 ± 0.4	3.5 ± 0.1	1.7 ± 0.5
42	F2016	5.3 ± 0.7	4.2 ± 0.9	1.1 ± 0.2
43	F2022	5.7 ± 0.5	4.9 ± 0.9	0.8 ± 0.1
45	F2021	5.1 ± 0.0	3.6 ± 0.6	1.5 ± 0.6
46	F2030	5.1 ± 0.1	3.8 ± 0.5	1.2 ± 0.4
47	F2029R	4.6 ± 0.4	3.3 ± 0.9	1.2 ± 0.1
49	Aubrey	5.7 ± 0.2	3.9 ± 1.0	1.7 ± 0.3
51	Ambassador	4.3 ± 0.7	2.8 ± 0.9	1.4 ± 0.0
52	Jupiter	5.3 ± 0.7	3.9 ± 0.8	1.3 ± 0.0
58	MSU Line F2010	4.4 ± 0.3	2.9 ± 0.2	1.4 ± 0.2
59	Unnamed 1	6.0 ± 0.3	4.8 ± 0.4	1.2 ± 0.6
60	Unnamed 2	5.3 ± 0.4	4.0 ± 1.3	1.3 ± 0.3
61	Unnamed 3	4.9 ± 0.2	3.9 ± 0.7	1.1 ± 0.5
62	F0013R	4.5 ± 0.3	3.6 ± 0.3	0.9 ± 0.0

[1] Results are expressed as milligrams of gallic acid equivalent per gram of sample, with the data presented as means ± standard deviation (n=3).

Table 14.3. Total Phenolic Acid Contents[1] of 2013 Wheat Fractions

Variety Number	Variety Name	Bran TPC (mg GAE/g)	Shorts TPC (mg GAE/g)	Flour TPC (mg GAE/g)
1	F1014	8.2 ± 0.5	7.5 ± 0.5	1.9 ± 0.7
2	F1026R	7.9 ± 0.2	6.0 ± 0.1	2.2 ± 0.6
3	F1012	7.3 ± 0.8	6.8 ± 0.4	2.3 ± 0.1
4	F1003R	8.5 ± 0.5	7.3 ± 0.1	1.8 ± 0.5
5	F1029	7.8 ± 1.1	8.0 ± 0.4	2.9 ± 0.1
6	F1047	8.4 ± 0.3	7.7 ± 0.4	2.5 ± 0.3
7	F1032R	9.1 ± 1.1	8.3 ± 0.3	2.2 ± 0.1
8	F1027	9.4 ± 0.3	5.6 ± 0.3	1.7 ± 0.3
9	F1049	8.6 ± 0.5	5.5 ± 0.9	2.0 ± 0.7
10	F1051	9.4 ± 0.9	9.5 ± 0.0	2.0 ± 0.3
11	F1050	8.2 ± 0.1	8.0 ± 0.4	2.7 ± 0.0
12	F1048	7.5 ± 0.5	7.4 ± 0.3	2.3 ± 0.2
13	F2031	8.6 ± 0.2	9.4 ± 0.3	1.5 ± 0.3
14	F2033	8.4 ± 0.0	8.9 ± 0.7	2.1 ± 0.2
15	F2034	10.4 ± 0.9	10.1 ± 0.5	2.4 ± 0.7
16	F2032	8.5 ± 0.3	7.0 ± 0.5	2.4 ± 0.7
17	F2037	9.0 ± 1.1	5.9 ± 0.5	2.6 ± 0.8
18	F2035	10.3 ± 0.0	5.0 ± 0.5	2.1 ± 0.9
19	F2041	9.4 ± 0.7	6.7 ± 0.1	1.6 ± 0.9
20	F2036	9.4 ± 0.0	8.0 ± 0.4	2.3 ± 0.4
21	F2038	7.9 ± 1.1	8.0 ± 1.0	1.3 ± 0.3
22	F2040	10.7 ± 0.7	7.0 ± 0.3	1.7 ± 0.4
23	F2042	9.4 ± 0.7	8.7 ± 0.7	2.1 ± 0.5
24	F2039	8.1 ± 0.1	7.7 ± 1.3	2.0 ± 0.6
25	F2001R	8.9 ± 0.5	8.0 ± 0.4	2.3 ± 0.8
26	F2006	8.1 ± 0.4	7.7 ± 1.2	2.1 ± 0.3
27	F2005	8.6 ± 1.0	8.6 ± 0.9	1.8 ± 0.7
28	F2015	9.2 ± 0.3	8.9 ± 1.6	2.4 ± 0.3
29	F2009	9.4 ± 0.6	8.8 ± 0.3	2.1 ± 0.7
30	F2012	8.8 ± 0.1	6.8 ± 1.2	2.1 ± 0.8
31	F2008	8.8 ± 0.2	7.3 ± 0.6	1.9 ± 0.5
32	F2011	13.0 ± 0.7	10.7 ± 0.4	3.3 ± 0.1
33	F2014R	8.6 ± 0.7	8.3 ± 0.5	2.0 ± 0.3
34	F2003	9.4 ± 0.4	8.5 ± 0.5	2.1 ± 0.3
35	F2004	7.6 ± 0.1	7.5 ± 0.4	1.7 ± 0.2
36	F2002	8.6 ± 0.4	7.9 ± 0.3	2.5 ± 0.6
37	F2028R	8.5 ± 0.5	8.4 ± 0.5	1.3 ± 0.6
38	F2020	6.9 ± 0.1	8.9 ± 1.0	1.4 ± 0.5
39	F2024R	10.2 ± 0.2	9.0 ± 0.8	1.8 ± 1.0
40	F2018	9.3 ± 0.3	9.1 ± 0.2	1.5 ± 0.9
41	F2019	9.0 ± 0.2	8.2 ± 0.5	2.1 ± 0.7
42	F2016	6.6 ± 0.1	8.7 ± 0.3	2.7 ± 0.7
43	F2022	11.3 ± 0.3	7.8 ± 0.2	2.9 ± 0.7

Table 14.3 (cont'd)

44	**F2025R**	**9.3**	**±**	**0.7**	**7.6**	**±**	**0.1**	**1.5**	**±**	**0.7**
45	F2021	10.4	±	1.4	9.4	±	0.3	2.9	±	0.9
46	F2030	11.9	±	0.3	10.5	±	0.2	2.4	±	0.8
47	F2029R	9.7	±	0.9	8.5	±	0.2	1.8	±	0.9
48	F2027R	9.6	±	0.1	7.7	±	0.3	2.1	±	0.7
49	Aubrey	9.6	±	0.3	8.1	±	0.7	2.0	±	0.6
50	Hopewell	9.4	±	0.2	7.6	±	0.2	2.7	±	0.9
51	Ambassador	8.1	±	0.2	6.1	±	0.4	1.6	±	0.8
52	Jupiter	9.1	±	0.2	8.9	±	0.5	2.4	±	0.7
53	Red Ruby	8.4	±	0.2	5.6	±	0.5	1.3	±	0.9
54	VA09W-188WS	8.4	±	0.3	7.6	±	0.1	1.5	±	0.8
55	Cayuga	8.3	±	0.1	8.7	±	0.2	2.1	±	0.5
56	VA09W-192WS	8.2	±	0.2	8.4	±	0.4	2.4	±	0.7
57	Caledonia	7.8	±	0.2	8.3	±	0.4	1.9	±	0.7

[1] Results are expressed as milligrams of gallic acid equivalent per gram of sample, with the data presented as means ± standard deviation (n=3).

Table 14.4. Total Phenolic Acid Contents[1] of 2014 Wheat Fractions

Variety Number	Variety Name	Bran TPC (mg GAE/g)	Shorts TPC (mg GAE/g)	Flour TPC (mg GAE/g)
2	F1026R	9.7 ± 0.4	7.9 ± 0.5	2.0 ± 0.3
5	F1029	10.4 ± 0.5	9.7 ± 1.1	2.3 ± 0.3
6	F1047	10.2 ± 0.4	8.1 ± 0.8	1.9 ± 0.2
8	F1027	9.1 ± 0.2	7.9 ± 0.7	1.9 ± 0.5
9	F1049	8.8 ± 0.8	7.3 ± 1.6	3.0 ± 0.3
12	F1048	11.5 ± 0.6	8.4 ± 0.8	2.6 ± 0.3
13	F2031	10.8 ± 0.1	8.7 ± 0.8	2.6 ± 0.2
14	F2033	9.8 ± 0.2	8.5 ± 1.0	2.7 ± 1.7
15	F2034	11.3 ± 0.4	8.0 ± 1.2	2.9 ± 1.1
17	F2037	9.1 ± 0.8	6.4 ± 1.2	2.2 ± 0.5
21	F2038	9.4 ± 0.5	9.7 ± 0.6	1.5 ± 0.2
23	F2042	9.8 ± 0.4	8.6 ± 1.0	2.5 ± 0.3
24	F2039	9.5 ± 1.0	10.2 ± 2.3	2.3 ± 0.0
27	F2005	9.7 ± 0.7	7.7 ± 0.4	2.1 ± 0.1
28	F2015	10.5 ± 0.0	8.9 ± 0.6	1.9 ± 0.5
29	F2009	9.5 ± 0.4	7.1 ± 0.2	2.0 ± 0.0
30	F2012	8.9 ± 0.3	7.7 ± 0.8	1.8 ± 0.8
31	F2008	9.5 ± 0.1	8.6 ± 0.8	2.2 ± 0.2
33	F2014R	9.4 ± 0.2	8.0 ± 1.4	2.7 ± 0.4
34	F2003	9.8 ± 0.3	7.5 ± 1.3	3.2 ± 0.3
36	F2002	10.4 ± 0.0	9.2 ± 0.8	2.3 ± 0.3
37	F2028R	10.0 ± 0.3	9.4 ± 0.1	2.4 ± 0.1
38	F2020	12.4 ± 0.9	8.6 ± 2.2	2.2 ± 0.5
39	F2024R	10.0 ± 0.5	8.7 ± 0.6	2.5 ± 0.2
40	F2018	10.4 ± 1.4	8.5 ± 1.6	2.2 ± 0.7
41	F2019	10.0 ± 0.5	8.1 ± 1.6	2.4 ± 0.3
42	F2016	10.1 ± 0.7	8.6 ± 0.8	2.1 ± 0.0
43	F2022	9.9 ± 0.2	8.6 ± 0.1	2.1 ± 0.9
45	F2021	9.7 ± 0.6	9.6 ± 0.3	2.5 ± 0.1
46	F2030	11.9 ± 0.3	8.2 ± 1.3	2.0 ± 0.2
47	F2029R	10.1 ± 0.6	9.3 ± 0.5	2.7 ± 0.6
49	Aubrey	9.5 ± 1.1	7.8 ± 0.2	2.6 ± 0.1
51	Ambassador	13.0 ± 1.5	8.0 ± 0.1	1.9 ± 0.6
52	Jupiter	11.1 ± 0.1	9.3 ± 0.3	2.1 ± 0.1
58	MSU Line F2010	9.7 ± 0.0	7.8 ± 0.0	1.7 ± 0.3
59	Unnamed 1	10.1 ± 0.7	8.7 ± 0.1	2.1 ± 0.2
60	Unnamed 2	10.5 ± 0.1	7.7 ± 0.3	3.1 ± 0.2
61	Unnamed 3	9.5 ± 0.2	7.5 ± 0.3	2.2 ± 0.2
62	F0013R	10.1 ± 0.4	9.1 ± 0.6	2.2 ± 0.0

[1] Results are expressed as milligrams of gallic acid equivalent per gram of sample, with the data presented as means ± standard deviation (n=3).

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