EFFECT OF GENOTYPE AND ENVIRONMENT ON NUTRITIONAL AND HEALTH BENEFICIAL COMPOUNDS IN WHEAT GRAIN

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COMPOUNDS IN WHEAT GRAIN

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NOMENCLATURE

ANOVA	Analysis of variance
GC	Gas chromatograph
FID	Flame ionization detector
PC	Policosanol
PS	Phytosterol
w/w	Weight to weight

Units

%	Percent
ºC	Degree Centigrade
g	Gram
hr	Hour
kg	Kilogram
mL	Millliliter
µL	Microliter
min	Minute
sec	Second
Ha	Hectare

CHAPTER 1

INTRODUCTION

1.1 STATEMENT OF PROBLEM

Wheat grain is rich in bioactive compounds, which provide nutritional and health benefits to humans and possess antioxidant properties. These compounds are concentrated in the outer layers of the grain, specifically in bran and germ fractions. Policosanol, a mixture of high molecular weight aliphatic primary alcohols, contribute to the health benefits of wheat grain. Policosanol content and composition of wheat bran samples obtained from 31 different wheat varieties grown in Oklahoma have been previously reported (Irmak and Dunford 2005), but the bran samples analyzed in the study were collected from a single location. Furthermore, determination of policosanol content only in the bran fraction might lead to misinterpretation of compositional variations among wheat varieties, because wheat varieties might have different milling properties resulting in varying amounts of flour carry-over into bran fractions. Since flour does not contain a significant amount of policosanol the analytical test results might be skewed depending on the amount of flour in bran fractions.

Wheat is also rich in phytosterols which have cholesterol lowering properties. The variation of phytosterol content and composition among wheat varieties has not been reported. The effect of environment on policasonol and phytosterol contents and compositions of wheat grain is also lacking.

1.2 HYPOTHESIS

Genotype and environment affect contents and compositions of nutritional and health beneficial bioactive compounds in wheat grain.

1.3 OBJECTIVES

The main objective of this thesis is to examine policosanol and phytosterol contents of whole grain samples obtained from three wheat varieties grown in three different locations in Oklahoma. The specific objectives include:

1) Examination of effect of genotype and environment on policosanol and phytosterol contents and compositions of whole wheat grain samples.
2) Study milling characteristics of Oklahoma grown wheat varieties.
3) Characterize protein, oil and ash content and mineral composition of whole wheat grain samples.

CHAPTER 2

REVIEW OF LITERATURE

2.1 OVERVIEW

2.1.1 HISTORICAL PERSPECTIVE

Wheat (Triticum aestium) is a wild grass native to arid countries of western Asia (Iqbal and others 2007). It originated in Southwest Asia in the area known as the Fertile Crescent. The first type of wheat cultivated was einkorn (Atwell 2001). Globally, wheat is an important human food ranking second in total cereal production behind maize with rice being the third. Wheat is largely used in daily life as a staple food and to make flour for leavened, flat and steamed breads, cookies, cakes, pasta and noodles. Due to the presence of a unique elastic protein complex, wheat is the only grain suitable for making leavened bread (Iqbal and others 2007). In addition, wheat is used to produce vinegar, white wine, beer, alcohol, vodka and biofuel through fermentation. Wheat is planted to a limited extend as a forage crop for livestock. Straw is used as fodder for livestock and construction material for roofing thatch.

2.1.2 WHEAT PRODUCTION

World wheat production was about 625 million metric tons in 2005 (FAO 2007). About 216 million Hectares (Ha) of wheat was harvested in the same year. The major producers of wheat are Europe, Pakistan, India, China, South and North America, and

Australia. China produces more wheat than any other country but it is also one of the largest importers.

In 2005, the US produced about 58.7 million tons of wheat on 202.4 million Ha. Wheat yield in the US was slightly higher than that of the world average, 2902 kg/Ha and 2896 kg/Ha, respectively. Wheat is the most important crop in Oklahoma (Epplin 1997). In 2005, about 3.5 million metric tons of wheat was produced on 2.3 million Ha. Although more wheat acres were planted in Oklahoma in 2007 (2.3 million Ha), production was significantly lower (2.7 metric tons) as compared to 2005. Because only 1.4 million Ha of the 2.4 million Ha of the planted wheat was harvested (USDA 2007).

2.1.3 WHEAT TYPES

There are three sets of terms to describe most modern wheat types. The first term, hard and soft, relates to the hardness of kernel. Hard wheat needs more energy to mill than soft wheat. The second term, red and white, refers to the presence or absence of a red pigment in the outer layers of the wheat kernel. The last term, winter and spring, describes the growth habit of the wheat (Atwell 2001).

The following wheat classes are used in the United States:

Durum wheat — Very hard, translucent, light colored grain. It is predominately used for producing semolina flour. Durum wheat is widely used for making high quality pasta products (Onyeneh and Hettiarachchy 1992).

Hard red spring wheat — Nearly all hard red spring wheat is grown in the northwest states in the US (Matz, 1991). Hard, brownish, high protein wheat used for bread and hard baked goods.

Hard red winter wheat — It is grown under widely varying environmental conditions (Matz 1991). Hard, brownish wheat used for bread, hard baked goods and as a blend with other flours to increase protein content of flour used for pie crusts. Some brands of unbleached all-purpose flours are commonly made from hard red winter wheat alone.

Soft red winter wheat —It is grown on smaller and diversified wheat farms (Matz 1991). Soft, low protein wheat used for cakes, pie crusts, biscuits, and muffins.

Soft white wheat — Soft, light colored, very low protein wheat grown in temperate moist areas. It is used for pie crusts and pastry.

Hard white wheat — Hard, light colored, opaque, chalky, medium protein wheat planted in dry and temperate climates. It is used for bread making and brewing.

In this study, we focus on three wheat varieties: Jagger, Intrada and Trego. Jagger is a hard red winter wheat, which was developed cooperatively by the Kansas Agricultural Experiment Station and the United States Department of Agriculture (USDA), Agricultural Research Service (ARS). It is a cross of KS82W418 and 'Stephens'. It has a high grain yield, strong disease resistance, and excellent bread-baking quality (Sears and others 1997). Jagger was released to seed producers in 1994.

Intrada is a hard white winter wheat developed cooperatively by the Oklahoma Agricultural Experiment Station (OAES), Kansas Agricultural Experiment Station and USDA-ARS, and released by the OAES and the USDA-ARS in September 2000. It is an F_3-derived line selected from the cross, 'Rio Blanco'/ 'TAM 200'. It has a high yield and test weight potential in the southern High Plains and for its end-use value in domestic and

export bread markets. Intrada performs best under dryland production conditions in western Oklahoma and in neighboring states (Carver and others 2003).

Trego, a hard white winter wheat, was developed cooperatively by the Kansas Agricultural Experiment Station and the USDA-ARS. It was released together by the Kansas, Nebraska, Colorado, and Oklahoma Agricultural Experiment Stations. It was selected from the cross KS87G325/ 'Rio Blanco' in 1988. Trego's winter hardiness is better than that of Jagger, and is recommended for dryland production throughout western Kansas. Trego has very good grain volume weight but lower flour ash and protein than Jagger (Martin and others 2001).

2.1.4 MILLING

Milling is simply the reduction of wheat grain to smaller particles that can be made into more palatable products. More specifically, it separates the germ and bran from the endosperm and reduces endosperm to flour. Generally, there are three steps involved in a dry milling process: cleaning, tempering, and milling. Cleaning removes undesirable components by classifying incoming material by size, shape, density, and magnetism. Then a predetermined amount of water is added and grain is tempered for a specific time period. The optimal tempering will bring the moisture to 13.5%-15.0% (w/w). This process may take 6-10 hours. In fact, this time varies considerably based on the hardness of the wheat. Temperatures lower than 50ºC are preferred during the tempering process (Atwell 2001). This process softens the grain and toughens the bran making it easier to separate it from the endosperm and germ. Also, tempering softens the endosperm which breaks apart with less force.

After cleaning and tempering, wheat is ready for milling. Roller mills are commonly used for wheat milling. Grain is sheared between two metal rolls which rotate in opposite directions. The germ is pliable and tends to flatten when it goes through the rollers. Bran particles form low-density small flakes. These properties allow millers to separate the germ and bran fractions from the endosperm fraction. A set of sieves separates the ground materials by size and density (Atwell 2001).

2.1.5 NUTRITIONAL BENEFITS

Whole grain foods are rich in fiber and provide complex carbohydrates, minerals, vitamins and phytochemicals. Some whole grain foods also contain omega-3 fatty acids, oligosaccharides and resistant starch (Anderson and others 2000). Because of its high nutritional value wheat became a leading cereal crop and important human diet in daily life, especially in developing countries. USDA specifically recommends consumption of 6 to 11 servings per day of grain products some of which should be from whole grains (Albertson and Tobelmann 1995). Numerous experimental and epidemiological studies have associated the intake of whole grains with reduced incidences of coronary heart disease (CHD) (Anderson and Hanna 1999; Liu and others 1999; Jensen and others 2004), diabetes (Meyer and others 2000; Liese and others 2003), and cancer (Chatenoud and others 1998; Nicodemus and others 2001). A meta-analysis of 12 studies showed that regular consumption of whole grain foods lowered the risk of CHD by 26% (Albertson and Tobelmann 1995). Total fiber intake was more effective than that of cereal fiber which contains refined grains. The bran fraction of grains, specifically wheat grain, contains numerous bioactive compounds including essential polyunsaturated fatty acids,

lignans which are potent phytoestrogens, antioxidants and other phytochemicals in addition to the fiber. All these compounds may act synergistically and contribute to the protective effect of total fiber.

Lignans in wheat bran provide protective effect against colorectal cancer (Qu and others 2005). Lignan is converted to enterodiol and enterolactone in the intestine. It is believed that these metabolites act as antioxidants and free radical scavengers, which may decrease the risk of cancer development. The same research group has also shown that lignan concentrations in wheat bran from 4 selected wheat cultivars were significantly different, but positively correlated with their antitumor activities (Qu and others 2005).

A study carried out using Min mice revealed an inverse relationship between the antioxidant content of the whole wheat and bran in diets and the number of intestinal tumors per mouse when dietary fiber content was equal (Carter and others 2006). In this study the antioxidant content of wheat varieties was determined by measuring orthophenolic compounds in both whole wheat and bran fractions. It was also reported that diets containing wheat bran were more effective in tumor suppression than whole wheat diets.

Wheat germ oil is rich in α-linolenic acid (omega-3) (2-9%). It has been shown that wheat germ oil supplementation of diet for patients with hypercholesterolemia reduced both oxidative stress and platelet formation (Alessandri and others 2006). Animal studies indicated that dietary administration of the lipid fraction of wheat bran significantly reduced the incidences of colon tumor formation (Reddy and others 2000). Wheat germ oil (WGO) feeding is shown to be effective in improving both fertility and successful delivery of golden hamsters (Soderwall and Smith 1962). Similar results were

obtained with breeder cows (Mariong 1962) and sheep (Dukelow and Matalamawki 1963).

The effect of WGO consumption on oxidative stress and platelet formation was investigated in a two-month clinical study including 32 patients with hypercholesterolemia (Alessandri and others 2006). Maize oil was used in control diets. Oxidative stress was measured by up-regulation of CD40 ligand (CD40L), a protein with inflammatory and prothrombotic properties. The study showed that WGO supplementation was effective in reducing both oxidative stress and platelet formation. Borel and others (Borel and others 1990) demonstrated that addition of wheat germ (WG) in diets fed to rats significantly decreased intestinal absorption of cholesterol. The authors postulated that phytosterols concentrated in WGO might have been the active compounds. Several other publications also reported cholesterol lowering properties of WGO (Ranhotra and others 1976; Hirai and others 1984).

It is well established that wheat and wheat products are rich in antioxidants and other health beneficial components. In 1999, the US Food and Drug Administration approved the following health claim. “Diets rich in whole-grain foods and other plant foods and low in total fat, saturated fat and cholesterol may reduce the risk for heart disease and certain cancers” (Anderson and others 2000). Unfortunately, the consumption of whole-grain foods by U.S. adults is below the recommended level (Cleveland and others 2000). A large proportion of the population could benefit from eating more whole grain, and efforts are needed to encourage consumption.

2.2 POLICOSANOL IN WHEAT

Policosanols (PC) are a mixture of high molecular weight (20-36 carbon) aliphatic primary long-chained alcohols. Docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), and triacontanol (C30) are the main PC in wheat varieties (Irmak and Dunford 2005; Irmak and others 2005). Although there is not enough scientific evidence to support the claim, it is believed that octacosanol (OC) is the compound with most activity (Taylor and others 2003). Only 5-20mg/day PC consumption may lower total cholesterol (TC) by 17-21% and low-density lipoprotein (LDL) by 21-29% and increase high-density lipoprotein (HDL) by 8-15% (Aneiros and others 1995; Gouni-Berthold and Berthold 2002). It is believed that PC inhibits cholesterol biosynthesis and increases LDL processing (Menendez and others 1994; Menéndez and others 2000). The efficacy of PC as a lipid-lowering agent has been studied extensively (Castano and others 1995; Mas and others 1999; Gouni-Berthold and Berthold 2002; Crespo and Alvarez 1997; Hernandez and others 1992; Varady and others 2003). PC at daily doses of 10 mg has been shown to have similar cholesterol lowering properties as the same dose of statins, simvastatin or pravastatin. Studies carried out for 3 or more years indicate that PC at a dose of up to 20 mg/day is safe and well tolerated (Gouni-Berthold and Berthold 2002). Beneficial effects of PC on smooth muscle proliferation and platelet aggregation have also been reported (Gouni-Berthold and Berthold 2002; Borg 1991). Snider examined the effect of octacosanol on Parkinson disease in a small controlled study (Snider 1984). The study indicated that although octacosanol may not be as potent as prescription anti-Parkinson medication, individuals with mild Parkinson disease may benefit from octacosanol consumption.

Ergogenic aids are the substances that enhance athletic performance by renewing or increasing energy stored in the body. WGO has been used as an ergogenic aid. WGO is reported to improve human physical fitness and this effect has been attributed to its high PC (Consolazio and others 1964; Cureton 1963). Dr. Cureton was the first to recognize the beneficial effect of WGO on athletic performance and he has worked twenty years to establish that WGO improved endurance and relieved stress. His research was compiled in a book entitled as "The Physiological Effects of Wheat Germ Oil on Humans in Exercise, Forty-two Physical Training Programs Utilizing 894 Humans" (Cureton 1972). In this book the beneficial effects of WGO and OC on a wide range of physical fitness related functions ranging from oxygen intake, breathe holding, and pulse rates to endurance were discussed.

PC formulations have been used as "antifatigue drugs" (Taylor and others 2003). One study carried out with tail-suspended rats indicated that OC could counteract simulated weightlessness effects on rats, suggesting that OC supplementation might benefit astronauts during space travels (Bai and others 1997).

The distribution of PC in wheat fractions was examined by Irmak and Dunford (Irmak and Dunford 2005). It was shown that PC is concentrated in bran and germ fractions of wheat grain. During wheat germ oil extraction PC is co-extracted. However, PC precipitates out of crude oil during cold storage. Conventional oil refining techniques also removes PC and these compounds end up in by-products. Irmak and others also studied the PC content and composition of 31 wheat varieties grown in Oklahoma (Irmak and Dunford 2005). Wheat grain samples were milled and bran fractions were used for analytical tests. The study showed that the Trego and Intrada varieties had the highest PC

content. Tetracosanol, hexacosanol and OC were the major PC components in all varieties. A recent study measured the PC content of Pegaso wheat lines grown in Kansas (Irmak and others 2007). There were significant differences among genotypes for PC content and composition. Both of the above studies were carried out using wheat bran fractions obtained from a pilot scale mill (Irmak and others 2007; Irmak and Dunford 2005). Bran samples used in these studies contained both germ fraction and a significant amount of flour due to low separation efficiency of pilot scale mills. Furthermore, different wheat varieties might lend themselves to milling differently causing variations in the amount of germ and flour residues in bran. Hence use of bran fractions for PC content determination might not be an accurate method to compare PC content in different wheat varieties. To eliminate this inaccuracy whole wheat grain samples were used for analytical tests in this study.

2.3 PHYTOSTEROL IN WHEAT

Phytosterols (PS), also called plant sterols, are cholesterol-like compounds and contain a four-ring steroid nucleus (Ostlund 2002). They differ in chemical structure from cholesterol by a side chain ethyl or methyl group. PS belong to the triterpene family of natural products (Moreau and others 2002). Most PS contain 28 or 29 carbons and one or two carbon–carbon double bonds, typically one in the sterol nucleus and sometimes a second in the alkyl side chain. Phytostanols are saturated PS (contain no double bonds). Sitosterol, stigmasterol and campesterol are the most abundant PS (Berger and others 2004; Ling and Jones 1995; Kritchevsky and Chen 2005). Brassicasterol, sitostanol and

campestanol are present in minor amounts in plants (Phillips and others 2002). PS may be present in the oil in free form and esterifies with glucosides, ferulic acid, or fatty acids.

PS are naturally found in vegetables, oilseeds, principally in oils, but also pulses and dried fruits (QuIlez and others 2003). Dietary sources of PS include corn, beans and plant oils (Ling and Jones 1995). In cereal grains they are mostly found in bran and are extractable as part of bran oil waxes (Wu and others 2007). Hence whole grains are better sources of PS than refined flour (Piironen and others 2000).

PS play major roles in pharmaceuticals, nutrition, and cosmetics. Since they cannot be synthesized in the human body, they must be supplied via one's diet. Early on, PS were used as pharmacological agents, but with the recognition that they are in a normal diet, they were added to conventional food products. Initially, PS were incorporated into margarines because of their higher solubility in lipids. As new and advanced delivery systems have been developed for incorporation of water insoluble PS into aqueous systems, fruit juice, ice cream and yogurt enriched in PS became available in grocery stores. PS have been declared GRAS (Generally Recognized as Safe) status by the US Food and Drug Administration (FDA). In the U.S., foods containing plant sterol esters can carry health claims. To qualify for the claim, a food must contain at least 0.65 g of plant sterol or 1.7 g of stanol esters per serving (Chapman 2000).

High serum concentrations of total or low-density-lipoprotein (LPL)-cholesterol are major risk factors for coronary heart disease and a major cause of mortality in developed countries (Plat and Mensink 2005; Tapiero and others 2003). Cholesterol lowering properties of PS were first demonstrated by Peterson in 1951 (Peterson 1951). Since then numerous studies confirmed beneficial effects of PS on human health.

The hypocholesteremic properties of PS have been studied extensively (Miettinen and others 2000; Jones and Ntanios 1998). A number of clinical studies demonstrated that PS can lower ‘‘bad’’ cholesterol (LDL) levels by as much as 10–14% in normal and hypercholesteromic adult males and females (with or without statin drugs), and children (Kritchevsky and Chen 2005; Plat and Mensink 2005; Lees and others 1977). Good cholesterol (HDL) levels and triacylglycerols were not affected. Another clinical study confirmed that patients already taking statin drugs could reduce their LDL levels an additional 10% by inclusion of PS consumption (Blair and others 2000).

Besides reducing cholesterol levels, PS may also provide protection against certain types of cancer such as colon, breast and prostate (Awad and Fink 2000; Tapiero and others 2003).

PS content and composition of wheat grain fractions and wheat germ oil have been reported by several research groups (Eisenmenger and Dunford 2007; Kiosseoglou and Boskou 1987; Jiang and Wang 2005; Itoh and others 1973; Seitz 1989; Hakala and others 2002; Nystrom and others 2007; Liu 2007). Wheat germ is a rich source of PS (2.4 mg/g) (Jiang and Wang 2005). The PS content of whole wheat (1.8 mg/g) and bran (1.2 mg/g) is lower than that of the germ fraction. Piironen and others (Piironen and others 2000) reported similar PS amounts in wheat bran (1.5–1.9 mg/g) but their study showed higher PS amounts in wheat germ (4.1 mg/g) than those of the reported by Jiang and Wang (Jiang and Wang 2005).

WGO contains a significantly higher amount of PS than do the other common commercial oils (Itoh and others 1973). PS constitute a major fraction of the WGO unsaponifiables (about 35%). Sitosterol (60-70%) and campesterol (20-30%) are the two

major PS present in WGO (Itoh and others 1973; Anderson and others 1926). The total PS content of WGO is about 3-4 % (Kiosseoglou and Boskou 1987). Esterified PS constitutes 2-3% of the oil. The majority of the PS in WGO is present in an esterified form (Kiosseoglou and Boskou 1987).

Jiang and Wang (Jiang and Wang 2005) reported that wheat bran and Durum wheat lipids have slightly lower levels of PS (17.7 and 15.1mg/g) compared with the wheat germ lipid sample (21.3 mg/g). Wheat fractions used in this study was obtained from a commercial milling operation. Eisenmenger and Dunford (Eisenmenger and Dunford 2007) showed that the wheat milling process, the wheat germ oil extraction process and oil refining techniques had a significant effect on the PS content of the final product. To the best our knowledge there is no comprehensive study examining variations of PS content and composition among wheat varieties. In this study we report PS content of three different wheat varieties.

2.4 OTHER WHEAT COMPONENTS

It is well known that the protein content of the grain affects the properties of processed wheat products. Crude protein content is often the most important factor influencing wheat price in world trade (Delwiche 1998). Wheat quality is often affected by genotype and environment (Graybosch and others 1995; Peterson and others 1992). The protein and ash contents of wheat flour vary between 9% and 14%, and 0.3% and 1.5%, respectively (Ferrao and Davanzo 2005). There appears to be no information on the lipid content of wheat varieties as affected by genotype and environment. This study will provide data on lipid content as well as protein, ash and mineral content of three wheat

varieties grown in three different locations in Oklahoma.

CHAPTER 3

MATERIALS AND METHODS

3.1 VARIETY SELECTION

In this study, three wheat varieties, Jagger, Trego and Intrada, were examined. Trego and Intrada varieties were chosen because a previous study carried out using bran fractions from 31 wheat varieties has shown that Trego and Intrada contained significantly higher PC than the other varieties examined in the study (Irmak and Dunford 2005). Jagger was included in the study because it is a popular variety in Oklahoma.

3.2 SAMPLE COLLECTION

Samples were collected from OSU variety testing program plots at Balko (100°07'W, 36°06'N), Goodwell (101°06'W, 36°06'N), and Alva (36°48′7″N, 98°39′57″W), OK. The test plots at Alva and Balko were both on farm fields, and the plots at Goodwell were on the Oklahoma State University Research Station. The plots were eight 15-cm rows wide by 12 m long, and agronomic management was performed according to Oklahoma State University extension recommendations. Plots were sown into a Ulysses silt loam (fine-silty, mixed, superactive, mesic Aridic Haplustolls) at Balko; into a Richfield silt loam (fine, smectitic, mesic Aridic Argiustolls) at Goodwell; and into a Grant silt loam at Alva. There were two sets of samples, irrigated and dryland, from the

Goodwell location. Planting dates for each location were as follows: Goodwell irrigated and dry-land: 10-01-2004, Balko: 10-18-2004, Alva: 09-30-2004. All fields were managed under "grain only" practices.

Whole wheat grain samples were collected after normal harvest. Most variety/locations had 3 replicates. Exceptions were Jagger at Alva, Trego at Balko and Jagger at Goodwell locations, which had 2, 4 and 4 replicates, respectively. Samples were stored in paper bags and kept in a freezer at -20°C after being received in our laboratory until our testing begun.

3.3 POLICOSANOL AND PHYTOSTEROL ANALYSIS

3.3.1 Sample preparation

Approximately 100 g of whole grain samples was brought to room temperature prior to grinding. The grain samples were ground 3 times in 30 seconds intervals using a coffee grinder (Black & Decker CBG5, Miami, FL) at medium speed. The coffee grinder was cleaned between each sample. The ground samples were stored in plastic Ziploc bags at -20°C until further analysis.

3.3.2 Hydrolysis

Approximately 2 grams of finely ground wheat grain sample was hydrolyzed by refluxing with 30 mL 1.0 N NaOH in methanol for 45 minutes while stirring. The solution was cooled and filtered through glass wool using a glass funnel. Residual material on the glass wool was washed with Millipore water and HPLC grade diethyl ether (Burdick & Jackson, Muskegon, MI). Then the filtrate was extracted with 20 mL

diethyl ether, and extraction was repeated three times. The diethyl ether layers combined from each extraction were washed with Millipore water until neutrality. The extract was dried at 45°C using a Reacti-Vap evaporation unit (Model 18780, Pierce, Rockford, IL) after drying over anhydrous sodium sulfate (ACS grade, EMD Chemicals Inc., Gibbstown, NJ). The residue was transferred to a 1ml volumetric tube. Then 250 μL silylation reagent [N-Methyl-N-(trimethylsilyl) trifluoroacetamide, (MSTFA), Pierce (Rockford, IL)] and 500 μL chloroform was added to the volumetric tube. The sample was derivatized by heating at 60°C for 15 minutes. The total volume was brought to 1mL by chloroform before analysis using a gas chromatograph (GC).

3.3.3 GC Analysis

PC and PS contents of the samples were analyzed by using a HP 6890 Series GC system. A fused silica capillary Equity-5 column (30 m x 0.25 mm x 0.5 μm film thickness) from Supelco (Bellefonte, USA) was used for the analysis. The oven temperature was programmed from 150°C to 320°C with 4°C/min heating rate and maintained at 320^{o}C for 15 minutes. Helium was used as carrier gas at a flow rate of 1.0 mL/min. The inlet temperature was 300°C. The samples (1 μL) were injected into the GC by an autosampler (HP 7683, HP Company, and Wilmington, DE). The split ratio was 1:10. The data collection and analysis were managed using an HP Chemstation (Rev. B.01.03 [204], Agilent Technologies, and Palo Alto, CA).

The PC and PS compositions of the samples were identified by direct comparison of their chromatographic retention times with those of authentic compounds. The individual PC standards used for peak identification, eicosanol (C20), heneicosanol (C21),

docosanol (C22), tricosanol (C23), tetracosanol (C24), hexacosanol (C26), heptacosanol (C27), octacosanol (C28) were purchased from Sigma (Sigma–Aldrich Corporation, St. Louis, MO) and used without further purification (97% and higher purity). Triacontanol (C30) (96%) was obtained from Aldrich (Sigma–Aldrich Corporation, St. Louis, MO). N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) from Pierce (Rockford, USA) was used as the derivatization reagent. The phytosterol standards were stigmasterol (95% purity), ß-sitosterol (97% purity) (Sigma-Aldrich Corporation, St. Louis, MO) and campesterol (Matreya Inc., Pleasant Gap, PA). Heptadecanol was used as internal standard. All other chemicals used in this study were reagent grade unless otherwise stated.

Stock solutions of PC and PS were prepared in chloroform (HPLC grade, Burdick & Jackson, Muskegon, MI) and derivatized with MSTFA at 60°C for 15-20 min. The desired concentrations of standard solutions were prepared by dilution of the stock solutions.

3.4 MILLING

Cleaned whole wheat grain samples were tempered to 15% moisture content overnight using a tempering instrument (GE A-G Gear Motor, MOD: 14PCP4265, Ford Wayne, IN). The grain samples were milled using a quadrumat senior mill (C.W. Brabender Instrument, INC, NJ). Each variety was milled separately, and the milling system was cleaned between varieties to avoid sample carryover. After milling, a sieve tester (Gilson Company, Inc, Ohio) was used to separate bran, break flour/shorts and flour fractions. The instrument was run for 5 min. Two U.S.A. standard testing sieves

(Seedburo Equipment Company, IL) with 500 and 150 μm openings were used in this process.

3.5 MOISTURE CONTENT

The moisture content of the samples was determined by AACC method number 44-15A (AACC, 1995). Ground wheat grain samples were brought to room temperature before analysis. Aluminum weighing dishes were pre-dried in a forced-air oven (VWR Scientific, Model 1370 FM, Bristol, CT) for an hour at 130°C before analysis. Then the aluminum dishes were kept in a desiccator to cool them to room temperature. About 2 grams of sample was weighed in the dried aluminum dish. Then the sample was dried in the oven at 130°C for 1 hr. The difference between the final and initial sample weight as percent of the initial sample weight was reported as the moisture content.

3.6 ASH CONTENT

The ash content of the wheat grain was determined according to the AOAC method 923.03 (AOAC, 1995). Ground wheat samples were brought to room temperature prior to use. Crucibles were pre-dried in a furnace (Fisher Scientific, Model 58 Isotemp® Muffle Furnace 600 Series, and Fair Lawn, NJ) for 5 hours at 525°C. The crucibles were cooled to room temperature in a desiccator. Approximately 2 g ground wheat grain samples were weighed into the dried crucibles and samples were ashed in a furnace for 5 hours at 525°C. The percentage residual weight in the crucibles was reported as the ash content in the sample.

3.7 LIPID CONTENT

Oil content was determined according to the AOAC method 960.39 (AOAC, 1995). Approximately 2 g of ground wheat sample was weighed into a cellulose thimble. The thimbles were then placed in the Soxtec extraction unit (Tecator, Model 1043 Extraction Unit, Sweden), and 40 mL of petroleum ether (Mallinckrodt, Paris, KE) was used to extract the oil from the sample. The extraction time was 30 min. The aluminum cup with the extracted oil was placed into vacuum oven (Fisher Scientific, Isotemp® Oven, Fair Lawn, NJ) for 15 min to evaporate the excess moisture. The amount of extracted oil was determined gravimetrically.

3.8 PROTEIN CONTENT

Protein is analyzed as nitrogen on a Leco TruSpec carbon-nitrogen analyzer (TruSpec CN, Leco USA, St. Joseph, MI) according to the method of Forage Analyses Procedures (Forage Analyses Procedures, 1993). A factor of 6.25 is used to convert nitrogen to protein.

3.9 MINERAL CONTENT

Ground wheat samples were ashed as described in section 3.6 of this thesis. Minerals were extracted from ash in hot 3 N HCl before analysis using an Inductively Coupled Plasma (ICP) spectrometer (Spectro Ciros, Fitchburgh, MA).

3.10 STATISTICAL ANALYSIS

All analytical tests were carried out at least in duplicate and in randomized order

with the mean values being reported. Analysis of variance (ANOVA) of the results was performed using the Mixed procedure of SAS for Windows (Software Version 9.1. SAS Institute Inc., Cary, NC). Environment was treated as a fixed effect.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 WHEAT GRAIN CHEMICAL COMPOSITION

4.1.1 Protein Content

Protein content of grain is well known to affect the functional properties of processed wheat products. Hence it is an important parameter analyzed to evaluate quality attributes of wheat varieties. Protein amount and composition in grain are significantly influenced by environmental conditions such as temperature and mineral availability to plant (Dupont and others 2006). This study examined protein contents of three different wheat varieties grown at three locations in Oklahoma. Effect of irrigation was examined at one location, Goodwell, OK. Average monthly temperatures and precipitation for each location are given in Appendix.

Protein contents of wheat varieties examined in this study varied from 10.1 to 17.9 % (Table 1). Wheat samples from Goodwell, dry-land (GWD) and Alva had the highest (>17 %, w/w) and lowest (about 10%, w/w) protein contents, respectively. Although the effect of environment on protein content was significant ($p < 0.05$), genotype and environment * genotype interaction were not. Samples from all locations except GWD, had protein content within the range reported in the literature (10-14%) (Ferrao and Davanzo 2005). Variations among the samples (standard error) were largest

at Balko. Differences in protein contents of the samples from GWD and Goodwell irrigated (GWI) were significant ($p < 0.05$).

4.1.2 Lipid Content

Although lipids are minor components of whole wheat grain they play important role in nutritional value, processing and storability of grain. Lipid content and composition of wheat are affected by variety and growth conditions (Konopka and others 2004). In wheat grain lipids are present in free and bound forms (Ruibal-Mendieta and others 2002).

Lipid contents of the wheat samples examined in this study are reported as weight percent of petroleum ether extracts (free lipids) based on whole grain weight. Table 2 shows lipid contents of the samples as affected by environment and genotype. Data reported in this thesis is slightly lower than the lipid contents of wheat grain samples reported by Ruibal-Mendieta and Konapka (Konopka and others 2004，Ruibal-Mendieta and others 2002). The variations might be due to different extraction method and solvents used for lipid recovery and moisture content of the samples. However, the results shown in Table 2 are within the range for lipid content in wheat varieties reported by Pomeranz (Pomeranz 1988).

Although the effects of environment and genotype on lipid content were significant environment * genotype interaction was not. Irrigation did not have a significant effect on oil contents of the samples at Goodwell location. Trego variety grown at Alva (1.39%) and Jagger at Goodwell (dry-land) (1.03%) had the highest and the lowest lipid contents, respectively. Wheat varieties grown in Alva had significantly

lower protein and higher lipid contents than the other varieties examined in this study (Table 1 and 2). A similar trend was also observed in soybean. Protein and lipid contents in soybean had a negative correlation (Kumar and others 2006).

4.1.3 Moisture and ash contents and mineral composition

Wheat samples were analyzed for their moisture content to complete proximate compositional data. It is important to know moisture content of the grain because it affects the milling process. Furthermore, high moisture content may promote microbial growth and chemical decomposition of wheat components during storage.

Table 3 shows moisture contents of the wheat samples examined in this study. As expected moisture contents of the samples varied between 11.9% and 13.1%. This data is similar to the wheat moisture content data published in literature (Sayaslan and others 2005).

Ash content which refers to the mineral content of grain varies with variety, agronomic practices such as fertilization and growth conditions (climate and soil type). Mineral composition of wheat grain affects flour extractability. Wheat can be an important and inexpensive source of certain minerals for humans in the developing countries.

Ash contents of the samples examined in this thesis are shown in Table 4. Intrada variety grown at Alva (1.62%) and Jagger grown at Goodwell (irrigated) (1.32%) had the highest and the lowest ash contents, respectively. Similar data for ash contents of wheat varieties were reported in the literature (Pomeranz 1988). Effects of environment and environment * genotype interaction were significant. However, genotype did not have a

significant effect on the ash content of wheat grain. Only Jagger variety grown on irrigated plots at Goodwell had significantly lower ash content than that of the grain grown on non-irrigated plots.

Ten minerals, P, Ca, K, Mg, Na, S, Fe, Zn, Fe and Mn, were identified in ash samples. Table 5 shows only 4 major minerals present in wheat grain. P and K contents of all the samples (varied between 2.9 mg/g and 4.8 mg/g) were higher than that of the other minerals. These concentrations are similar to the data reported by Pomeranz (Pomeranz 1988). Significant amount of Mg and Ca were also detected in the samples.

4.2 MILLING FRACTIONS

Genotype and environment have a significant effect on milling characteristics of wheat grain and flour extraction rates (Peyron and others 2003). Wheat grain milling behavior is very important from processing and economic point of view. Wheat samples examined in this study were milled using a quadrumat senior mill as explained in chapter 3, section 3.4 in detail. Three fractions, bran, break flour (BF) and flour + shorts (FS) were obtained by sieving. About 50-55% of the grain consisted of FS fraction (Table 6). Bran was the second largest fraction, 26-33% of the grain. Although effect of environment on the amount of bran collected was significant genotype and genotype * environment interaction were not. Irrigation was also a significant factor affecting the amount of bran collected during the milling process.

4.3 POLICOSANOL CONTENT IN WHEAT GRAIN

Literature on PC contents and compositions of wheat varieties are limited. In a

previous study carried out in our research group PC contents and compositions of 31 wheat varieties grown in Oklahoma were examined (Irmak and Dunford 2005). The study focused on bran fraction of the grain and samples were collected from a single location. This thesis examined samples from 3 varieties grown at 3 different locations. Effects of irrigation and dry-land conditions on PC contents and compositions of wheat varieties were also investigated. The reason for using whole wheat grain in this study was to eliminate the errors that might be introduced by inconsistent flour carryover in bran fraction during milling operation.

PC contents of the samples varied between 15.9 mg/kg and 28.7 mg/kg (Table 7). These values are lower than the data reported in the previous study (Irmak and Dunford 2005). This is mainly due to the fact that the current study used whole grain rather than bran. Whole grain contains higher amounts of endosperm (flour) which does not contain significant amount of PC (Irmak and Dunford 2005). Intrada grown at Balko had the highest PC content (28.7 mg/kg) among the samples. The same variety was identified as one of the two high PC content varieties in the previous study (Irmak and Dunford 2005). Total PC contents of bran from an Italian bread variety Pegaso and its 11 near-isogenic lines were significantly lower than that of the varieties examined in this study (Irmak and others 2007).

Effects of environment, genotype and environment * genotype interaction on PC content were very significant. Standard error for Balko and Alva locations were larger than that of Goodwell location. Irrigation had a significant effect on PC contents. It appears that dry-land conditions favored PC synthesis in the plant. PC is a group of compounds that are classified as wax. One of the functions of wax is to preserve the

water balance of the plant. Higher amounts of PC synthesized under dry-land conditions might be due to a defense mechanism in the plant to minimize moisture loss for survival. However, this hypothesis needs to be tested by further experiments. C23, C24, C26, C28 and C30 were the most abundant PC components detected in all the samples (Table 8).

4.4 PHYTOSTEROL CONTENT IN WHEAT GRAIN

Wheat contains a significant amount of PS which have cholesterol lowering properties and used in many functional food and nutraceutical formulations. Although PS contents and compositions of wheat milling fractions have been examined by several groups (Jiang and Wang 2005; Nystrom and others 2007; Hakala and others 2002), effects of genotype and environment on PS content and composition in whole wheat grain have not been reported.

Table 9 shows total PS content of wheat samples as affected by genotype and environment. Jagger variety grown at Balko had the highest total PS content, 359.7 mg/kg. Jiang and Wang (Jiang and Wang 2005) reported higher total PS content in Drum wheat. However, it should be noted that authors reported total PS amount in wheat extracts rather than whole wheat. Nystrom and others (Nystrom and others 2007) reported PS content in whole wheat (783 mg/kg), which was slightly higher but the same order of magnitude as the data reported in this thesis (205.8-359.7 mg/kg). The reasons for the differences might be two folds. Nystrom study used both acid and alkaline hydrolysis for PS analysis in the samples. In this thesis only alkaline hydrolysis was carried out. The second reason might be due to variations in variety and crop growth conditions in two studies. Seitz (Seitz 1989) reported much lower total PS content for

several wheat varieties. The discrepancy can be explained by different analytical protocols, varieties and agronomic practices used in these studies.

In the current study environment, genotype and genotype * environment interaction had a significant effect on the PS contents of the wheat samples. Samples harvested from Goodwell had higher standard error than the samples collected from Alva and Balko. Effect of irrigation on PS content was significant for Trego and Jagger but not for Intrada variety.

β-Sitosterol, campesterol and stigmasterol were the major PS found in all the samples (Table 10). The most abundant phytosterol was β-sitosterol.

CONCLUSIONS

This study examined effects of genotype, environment and irrigation on nutritional and health beneficial wheat grain components and bran separation efficiency. Protein, lipid, ash, mineral, PC and PS contents and compositions of whole wheat grain samples were also analyzed.

Protein contents of the samples varied between 10.1% and 17.9% (w/w). Effect of environment on protein content was significant.

Lipid contents of the wheat samples were between 1.03% and 1.39% (w/w). The data reported in this study was similar to that published in the literature. Effects of environment, genotype and irrigation on lipid content in wheat grain were significant.

Wheat grain samples had ash content in the range of 1.23% to 1.62%, which were similar to the data published in the literature. Effects of environment and environment * genotype interaction on ash content were significant. P, K, Mg and Ca were the major minerals found in all the samples.

About 50 to 55% of the grain consisted of flour + shorts. Bran (26-33%) was the second largest fraction obtained from milling. Environment and irrigation had significant effects on efficiency of bran recovery from wheat grain.

PC contents of the whole wheat grain samples were between 15.9 mg/kg and 28.7 mg/kg. Environment, genotype, genotype * environment interaction and irrigation had

significant effects on total PC contents of the samples. Dry-land conditions favored higher PC synthesis in the grain.

Whole wheat grain samples had PS contents between 205.8 mg/kg and 359.7 mg/kg. Environment, genotype and environment * genotype interaction had significant effects on PS contents of the samples. Effect of irrigation on PS content was significant only for Trego and Intrada varieties. β-Sitesterol, campesterol and stigmasterol were the major PS components in all the samples.

To the best of our knowledge this is the first extensive and systematic study examining the effect of environment, genotype and irrigation on nutritional and health beneficial components of whole wheat grain samples. This study clearly indicates that agronomic practices, climate and variety have significant effects on the health beneficial wheat grain components. A fundamental understanding of compositional variations in grains requires extensive research on effects of genotype and environment over several years. This study is the first step achieving this goal.

FUTURE WORK

Wheat grain is an excellent staple food containing numerous nutritional and health beneficial compounds. This study focused on protein, lipid, mineral, PS and PC contents of 3 wheat varieties. It is also important to examine variations in concentrations and compositions of lignan, natural antioxidants and other bioactive compounds naturally present in wheat. Verification of variations in compositional data over several years is also crucial. This line of research will lead to elucidation of biological pathways that are involved in synthesis of bioactive components in wheat and ultimately help to engineer new wheat varieties that can be used as biorefineries to produce these valuable compounds in large quantities. Development of new processes that will aid recovery and/or concentration of health beneficial wheat components in value-added product such as functional foods and nutraceuticals will benefit consumers, processors and farmers by providing healthy food choices and finding new applications for wheat grain and its by-products.

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Table1: Protein contents of wheat grain samples [% (w/w), as is basis].

Variety \ Location	Alva	Balko	GWD*	GWI**
Trego	10.1 ± 0.8	12.1 ± 1.5	17.9 ± 0.4	14.0 ± 0.6
Jagger	10.6 ± 0.6	12.8 ± 2.0	17.2 ± 0.7	14.2 ± 0.9
Intrada	10.2 ± 0.2	12.2 ± 1.4	17.2 ± 0.5	14.1 ± 1.3

* Goodwell-dryland
**Goodwell-irrigated

Table 2: Lipid contents of wheat grain samples [% (w/w), as is basis].

Variety \ Location	Alva	Balko	GWD*	GWI**
Trego	1.39 ± 0.02	1.22 ± 0.08	1.3 ± 0.2	1.19 ± 0.03
Jagger	1.27 ± 0.02	1.2 ± 0.1	1.03 ± 0.07	1.20 ± 0.06
Intrada	1.32 ± 0.06	1.13 ± 0.03	1.14 ± 0.07	1.22 ± 0.05

* Goodwell-dryland
**Goodwell-irrigated

Table 3: Moisture contents of wheat grain samples [% (w/w), as is basis].

Variety \ Location	Alva	Balko	GWD*	GWI**
Trego	12.5 ± 0.3	12.8 ± 0.5	12.2 ± 0.6	12.2 ± 0.5
Jagger	12.4 ± 0.08	12.0 ± 0.5	11.9 ± 0.2	12.2 ± 0.4
Intrada	12.6 ± 0.4	13.1 ± 0.4	12.0 ± 0.2	12.5 ± 0.4

* Goodwell-dryland
**Goodwell-irrigated

Table 4: Ash contents of wheat grain samples [% (w/w), as is basis].

Variety \ Location	Alva	Balko	GWD*	GWI**
Trego	1.52 ± 0.03	1.34 ± 0.05	1.52 ± 0.02	1.51 ± 0.03
Jagger	1.57 ± 0.04	1.35 ± 0.07	1.46 ± 0.07	1.32 ± 0.05
Intrada	1.62 ± 0.04	1.36 ± 0.02	1.41 ± 0.05	1.42 ± 0.06

* Goodwell-dryland
**Goodwell-irrigated

Table 5: Mineral compositions of wheat grain samples (mg/g, as is basis).

Variety/Location/Mineral		Alva	Balko	GWD*	GWI**
Trego	**P**	3.3 ± 0.08	2.9 ± 0.2	3.3 ± 0.06	3.2 ± 0.05
	Ca	0.4 ± 0.01	0.5 ± 0.07	0.5 ± 0.02	0.4 ± 0.05
	K	4.4 ± 0.4	3.5 ± 0.2	3.7 ± 0.09	3.8 ± 0.09
	Mg	1.2 ± 0.03	1.3 ± 0.1	1.4 ± 0.04	1.4 ± 0.02
Jagger	**P**	3.4 ± 0.2	3.0 ± 0.2	3.1 ± 0.4	3.0 ± 0.2
	Ca	0.4 ± 0.07	0.5 ± 0.06	0.5 ± 0.03	0.4 ± 0.03
	K	4.4 ± 0.2	3.5 ± 0.1	3.9 ± 0.3	3.8 ± 0.2
	Mg	1.3 ± 0.02	1.4 ± 0.06	1.3 ± 0.1	1.3 ± 0.07
Intrada	**P**	3.6 ± 0.2	3.1 ± 0.2	3.0 ± 0.1	3.1 ± 0.2
	Ca	0.4 ± 0.07	0.5 ± 0.03	0.6 ± 0.06	0.5 ± 0.03
	K	4.8 ± 0.4	3.4 ± 0.09	3.4 ± 0.2	3.6 ± 0.2
	Mg	1.4 ± 0.1	1.4 ± 0.04	1.4 ± 0.04	1.4 ± 0.09

* Goodwell-dryland
**Goodwell-irrigated

Table 6: Weight percent of wheat milling fractions collected as bran (particle size > 500 μm), break flour (particle size between 500 μm and 100 μm) and Flour + shorts (particle size < 100 μm).

Variety	Location	Alva	Balko	GWD*	GWI**
Bran	**Trego**	31.8 ± 3.4	28.5 ± 2.0	29.8 ± 2.2	27.5 ± 1.7
	Jagger	33.0 ± 0.3	28.4 ± 1.6	30.3 ± 0.6	25.7 ± 0.6
	Intrada	32.2 ± 0.7	29.3 ± 2.3	31.1 ± 1.6	26.0 ± 1.3
Break Flour	**Trego**	16.4 ± 4.1	22.5 ± 3.4	23.8 ± 2.4	20.6 ± 1.8
	Jagger	15.9 ± 0.3	20.6 ± 1.2	19.1 ± 1.2	22.7 ± 7.8
	Intrada	14.8 ± 1.4	19.3 ± 2.2	20.7 ± 0.8	20.5 ± 1.3
Flour + Shorts	**Trego**	53.0 ± 1.8	50.2 ± 1.9	48.6 ± 0.8	55.4 ± 0.8
	Jagger	54.0 ± 0.06	53.3 ± 1.2	53.3 ± 0.9	53.7 ± 6.7
	Intrada	55 ± 1	52.8 ± 0.5	49.7 ± 1.1	55.3 ± 0.4

* Goodwell-dryland
**Goodwell-irrigated

Table 7: Total policosanol contents of wheat grain samples as affected by genotype and environment (mg/kg whole grain, as is basis).

Variety \ Location	Alva	Balko	GWD*	GWI**
Trego	26.8 ± 3.5	22.3 ± 3.1	27.2 ± 2.2	21.4 ± 1.6
Jagger	16.3 ± 0.9	24.4 ± 1.4	23.1 ± 1.6	15.9 ± 1.2
Intrada	26.2 ± 2.5	28.7 ± 3.8	20.6 ± 1.5	16.0 ± 1.4

* Goodwell-dryland
**Goodwell-irrigated

Table 8: Policosanol compositions of wheat grain samples as affected by genotype and environment (mg/kg, whole grain, as is basis).

Variety/Location/PC		Alva	Balko	GWD	GWI
Trego	**C23**	10.0 ± 1.8	6.3 ± 1.2	10.2 ± 2.2	7.5 ± 1.0
	C24	5.7 ± 1.4	6.1 ± 1.2	7.4 ± 0.9	6.2 ± 1.1
	C26	2.8 ± 0.5	3.2 ± 0.5	3.4 ± 0.5	1.9 ± 0.5
	C28	1.4 ± 0.3	0.7 ± 0.2	1.0 ± 0.4	1.9 ± 0.3
	C30	3.6 ± 0.6	3.1 ± 0.4	2.4 ± 0.3	2.1 ± 0.4
Jagger	**C23**	6.5 ± 1.2	8.5 ± 0.8	9.6 ± 1.3	6.2 ± 0.9
	C24	3.5 ± 0.4	6.2 ± 1.0	5.6 ± 0.7	4.7 ± 0.3
	C26	2.1 ± 0.4	3.2 ± 0.4	2.0 ± 0.3	1.2 ± 0.2
	C28	0.93 ± 0.07	0.8 ± 0.3	2.0 ± 0.5	0.57 ± 0.06
	C30	1.2 ± 0.3	2.6 ± 0.4	2.0 ± 0.5	1.6 ± 0.3
Intrada	**C23**	11.1 ± 2.4	10.9 ± 1.6	5.6 ± 0.8	3.4 ± 0.5
	C24	5.5 ± 0.7	7.5 ± 1.6	4.7 ± 0.8	3.9 ± 0.7
	C26	2.8 ± 0.3	3.3 ± 0.4	2.3 ± 0.4	2.6 ± 0.8
	C28	0.9 ± 0.2	0.8 ± 0.2	0.6 ± 0.1	0.9 ± 0.2
	C30	3.1 ± 0.8	3.6 ± 0.8	4.4 ± 0.6	2.6 ± 0.3

(C23=Ticosanol, C24=Tetracosanol, C26=Hexacosanol, C28=Octacosanol, C30=Triacontanol)

* Goodwell-dryland
**Goodwell-irrigated

Table 9: Total phytosterol contents of wheat grain samples as affected by genotype and environment (mg/kg whole grain, as is basis).

Variety \ Location	Alva	Balko	GWD*	GWI**
Trego	250.4 ± 25.9	310.7 ± 21.8	314.8 ± 24.2	201.5 ± 23.1
Jagger	205.8 ± 20.4	359.7 ± 28.2	253.0 ± 39.3	243.9 ± 21.9
Intrada	237.5 ± 29.5	318.8 ± 16.8	244.9 ± 24.6	240.7 ± 28.2

* Goodwell-dryland
**Goodwell-irrigated

Table 10: Phytosterol compositions of wheat grain samples as affected by genotype and environment (mg/kg, whole grain, as is basis).

Variety/Location/PC		Alva	Balko	GWD	GWI
Trego	**β- sitosterol**	181.7 ± 14.8	252.2 ± 20.7	259.1 ± 24.0	150.1 ± 22.5
	Stigmasterol	8.0 ± 0.8	8.4 ± 1.6	9.4 ± 1.0	6.2 ± 1.2
	Campesterol	42.6 ± 8.7	49.9 ± 4.6	46.7 ± 5.7	45.2 ± 5.5
Jagger	**β- sitosterol**	150.2 ± 20.5	282.7 ± 20.8	259.1 ± 24.0	150.1 ± 22.5
	Stigmasterol	8.5 ± 0.8	8.4 ± 1.6	9.4 ± 1.0	6.2 ± 1.2
	Campesterol	47.0 ± 2.4	49.9 ± 4.6	46.7 ± 5.7	45.2 ± 5.5
Intrada	**β- sitosterol**	189.9 ± 22.6	259.1 ± 13.6	201.3 ± 21.9	195.1 ± 24.3
	Stigmasterol	9.1 ± 1.9	9.9 ± 1.2	8.7 ± 0.8	6.82 ± 0.9
	Campesterol	38.5 ± 7.8	47.1 ± 6.0	34.9 ± 4.9	240.7 ± 28.2

* Goodwell-dryland
**Goodwell-irrigated

Figure 1: A typical gas chromatogram of a mixture of PC and PS standards.

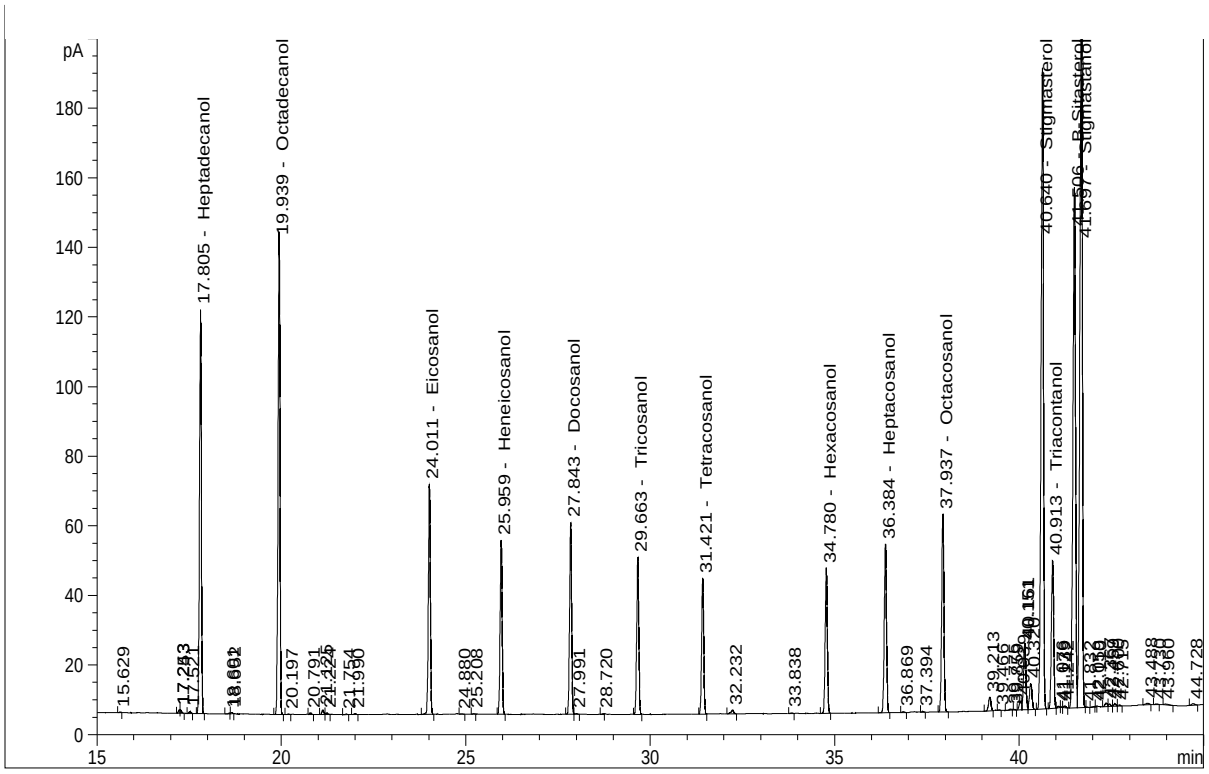
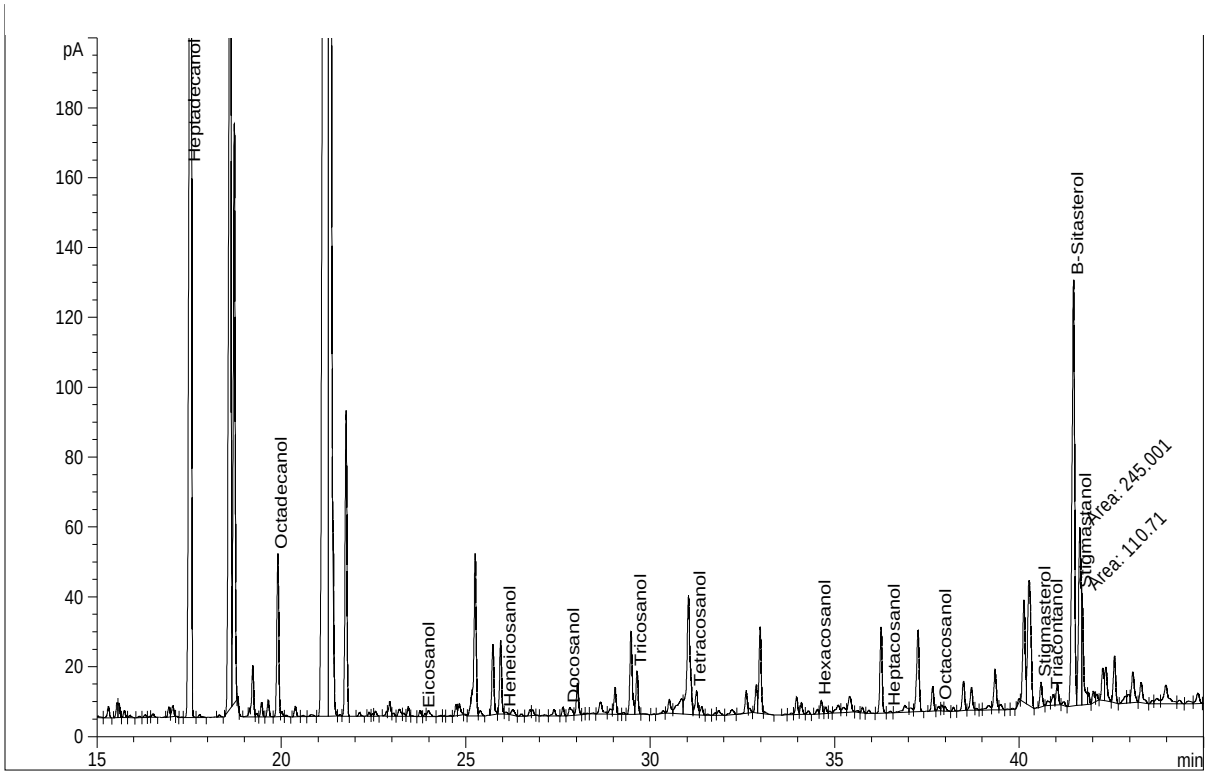


Figure 2: A typical gas chromatogram of wheat grain extracts.



APPENDIX

Figure 3: Average monthly temperatures (ºC) at Alva.

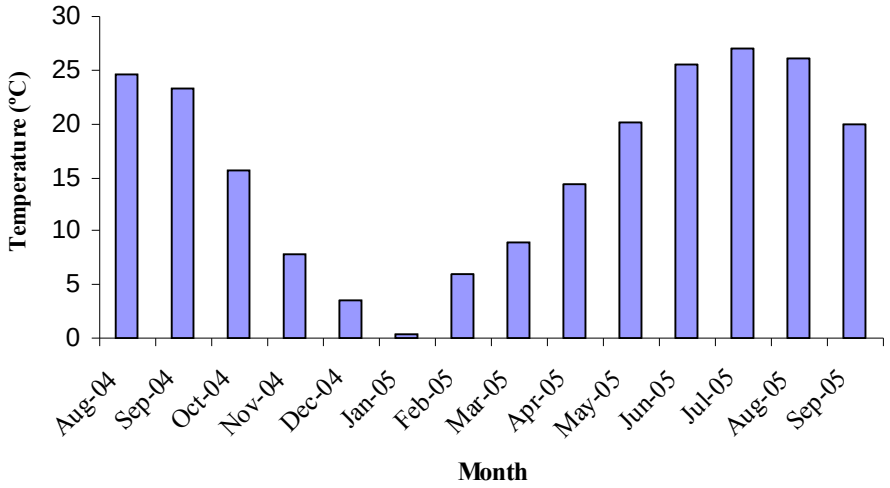


Figure 4: Total monthly rainfall (cm) at Alva.

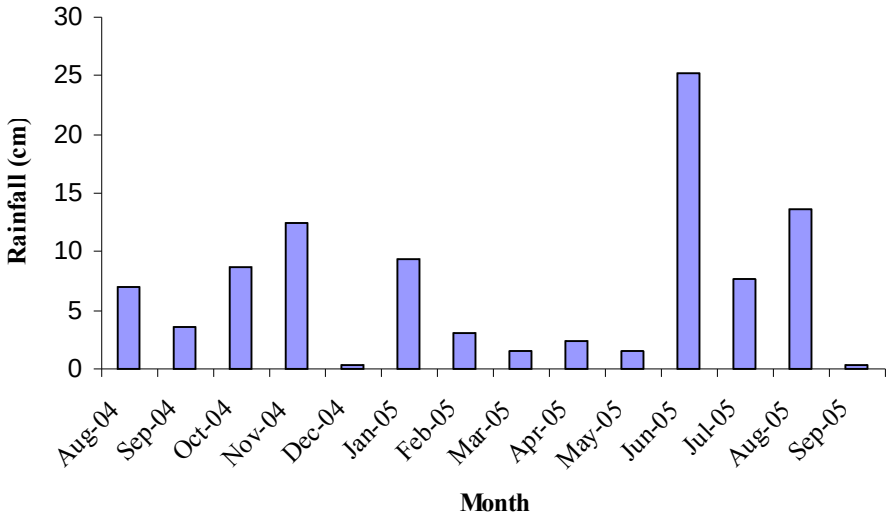
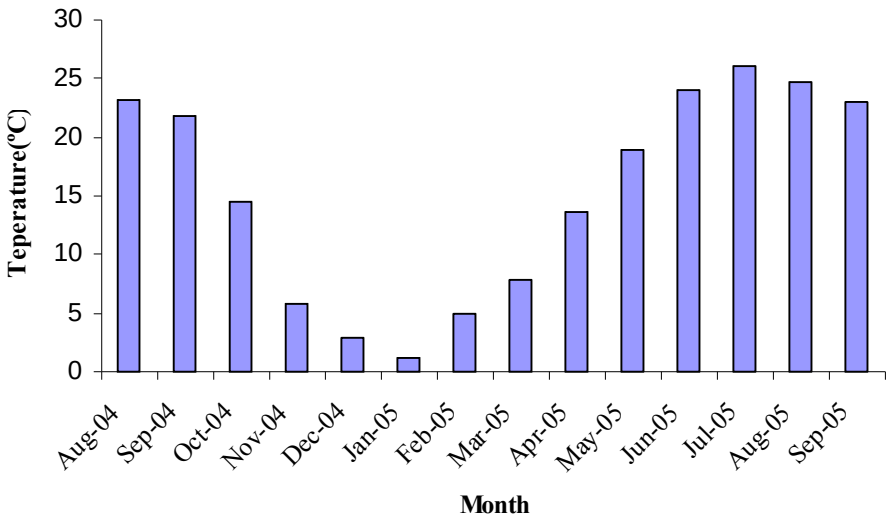
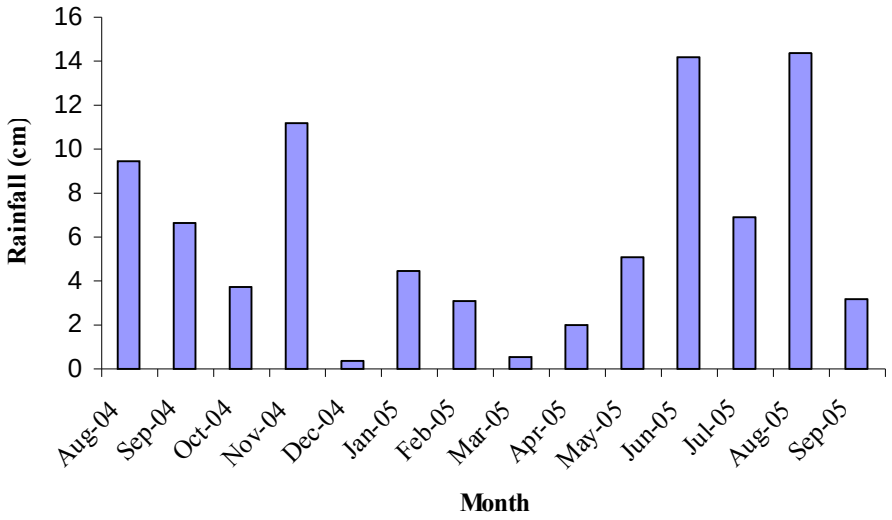


Figure 5: Average monthly temperatures (ºC) at Beaver*.



* Since there is no weather station at Balko, the data from Beaver which is the closest station (approx 10 miles away) to Balko is shown.

Figure 6: Total monthly rainfall (cm) at Beaver*.



* Since there is no weather station at Balko, the data from Beaver which is the closest station (approx 10 miles away) to Balko is shown.

Figure 7: Average monthly temperatures (ºC) at Goodwell.

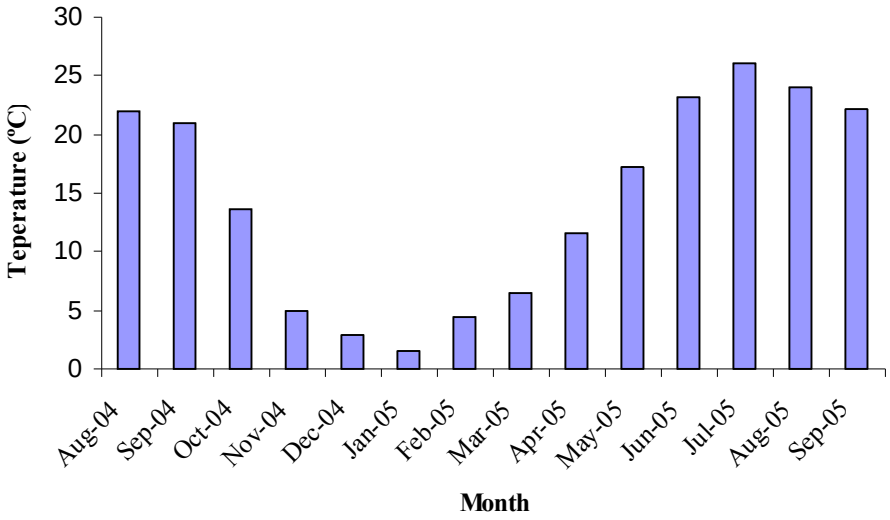
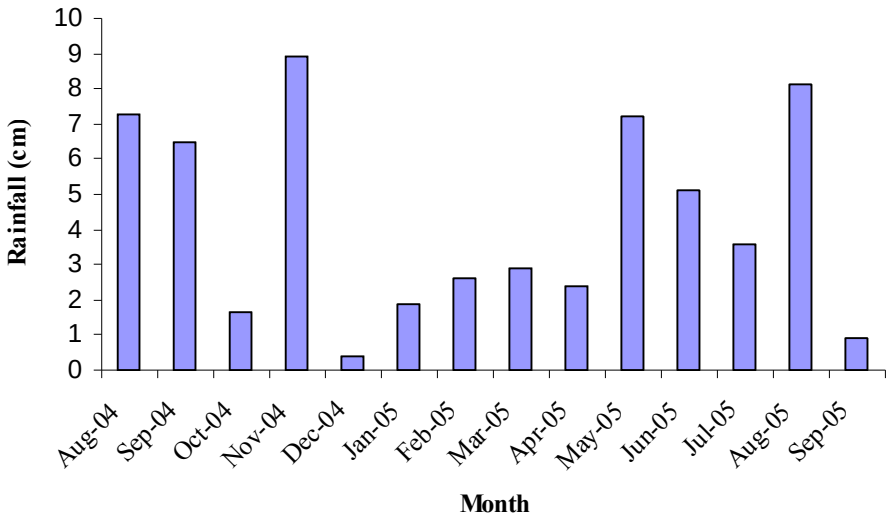


Figure 8: Total monthly rainfall (cm) at Goodwell.



VITA

YONGFEN CHEN

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF GENOTYPE AND ENVIRONMENT ON NUTRITIONAL AND HELTH BENEFITIAL COMPOUNDS IN WHEAT GRAIN

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Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: Effect of genotype and environment on nutritional and health beneficial compounds in wheat grain

Pages in Study: 65 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: The main objective of this study was to evaluate effect of genotype and environment on nutritional and health beneficial wheat grain components. Three wheat varieties, Jagger, Trego and Intrada were used in this research. The samples were collected from three locations, Alva, Balko and Goodwell, Oklahoma. In Goodwell, there were two different agronomic practices, dry-land and irrigated. Total policosanol and phytosterol contents and compositions in whole wheat grain samples were determined. Milling characteristics of the same samples were evaluated. Protein, ash, moisture, lipids and mineral contents of the samples were also analyzed.

Findings and Conclusions: This study clearly indicates that genotype, environment and irrigation have significant effects on the health beneficial wheat grain components. Dry-land conditions favored higher protein and policosanol synthesis in wheat. A fundamental understanding of effect of genotype and environment on wheat grain chemical composition requires extensive research over several years. This study is the first step toward achieving this goal.

ADVISER'S APPROVAL: Nurhan T. Dunford