EFFICIENCY OF ABRASIVE DEHULLING TO PRODUCE WHEAT GRAIN FRACTIONS ENRICHED IN ANTIOXIDANTS

By

YONGFEN CHEN

Bachelor of Science in Food Science
China Agricultural University
Beijing, China
2004

Master of Science in Food Science
Oklahoma State University
Stillwater, Oklahoma
2007

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Dissertation Approved:

Nurhan. T. Dunford

Dissertation Adviser

Mark Wilkins

Jeff Edwards

Carla Goad

Dr. Mark E. Payton

Dean of the Graduate College

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NOMENCLATURE

HPLC	High performance liquid chromatography
GC	Gas chromatography
FID	Flame ionization detector
PC	Policosanol
PS	Phytosterol
w/w	Weight to weight

Units

%	Percent
ºC	Degree Centigrade
g	Gram
h	Hour
kg	Kilogram
mL	Milllliliter
µL	Microliter
min	Minute
s	Second
oz	Ounce

CHAPTER1

INTRODUCTION

1.1 STATEMENT OF PROBLEM

It is well established that wheat is rich in nutritional and health beneficial bioactive compounds, specifically compounds with high antioxidant capacity. These compounds are not uniformly distributed in the wheat kernel. They are particularly concentrated in the outer layers of the grain comprising bran and germ. Although endosperm or flour which is rich in starch has been widely used in food and industrial bioproducts, wheat milling industry by-products, germ and bran, have not been exploited to their full capacity for value-added products development.

The traditional wheat milling process was designed to refine flour and remove bran and germ as byproducts. In general, conventional roller mills use sieving and air separation for flour recovery, resulting in significant flour residue in bran and germ fractions. Since flour does not contain substantial amounts of bioactive compounds, flour contamination dilutes bioactive compounds in bran and germ fractions. Development and optimization of milling techniques that will efficiently separate bran and germ and minimize flour residue in these fractions are necessary to improve the economic feasibility of recovery of health beneficial compounds from whole wheat grain.

Tangential abrasive dehulling technique was developed for controlled removal of outer layers of grains from endosperm. This method has not been optimized for

separation of bioactive wheat components from whole grain. To the best of my knowledge there is no study correlating chemical composition of wheat grain fractions to their antioxidant capacity. An understanding of the correlations between chemical composition and antioxidant capacity of wheat grain fractions is critical for designing efficient downstream processes to be used for value-added products development. Furthermore, formulation of nutraceutical products, such as policosanol and vitamin E, for specific health conditions entails the knowledge of relationship between chemical composition and the efficacy of the products.

1.2 HYPOTHESIS

Tangential abrasive dehulling is an efficient method to produce wheat grain fractions enriched in health beneficial bioactive compounds.

1.3 OBJECTIVES

The objective of this dissertation is to examine the efficiency of the tangential abrasive dehulling method to produce wheat fractions enriched in health beneficial bioactive compounds. The specific objectives include:

1) To study tangential abrasive dehulling process for producing wheat grain fractions enriched in bioactive compounds with high antioxidant capacity.

2) To determine the chemical compositions and antioxidant capacity of wheat fractions obtained by Tangential Abrasive Dehulling Device (TADD), whole wheat, bran and commercial aleurone products.

3) To develop correlations between antioxidant capacity and the chemical compositions of TADD and commercial products.

CHAPTER 2

REVIEW OF LITERATURE

2.1 INTRODUCTION

Wheat is a primary agricultural product not only in the United States but worldwide. It is the main food source in many diets. In 2008, world wheat production reached 690 million metric tons with China, India, and the U.S. being the top three largest producers (FAO 2010). Wheat, as the primary cereal grain, comprises up to one third of the world grain production (Slavin and others 2000).

In 2007, wheat production ranked fourth after cow milk, maize and sorghum in U.S. (USDA 2010a). Oklahoma is one of the major wheat producers in the U.S. (USDA 2008). According to 2009 State Agriculture Overview of Oklahoma, wheat production ranked third among all the crops producing 77,000,000 bushels and brought $ 369,600,000 to the state (USDA 2010b). However, in the past ten years, wheat production has decreased. Oklahoma is an important provider of winter wheat in the U.S. (USDA 2008). Hard winter wheat is the primary winter type in Oklahoma and there are more than 40 varieties of hard winter wheat grown in Oklahoma (USDA 2010c). These varieties include Jagger, Trego, and Intrada.

A study of 31 wheat varieties grown in Oklahoma showed that Trego and Intrada varieties had the highest amount of policosanol (PC), a group of compounds with low

density lipoprotein (LDL) lowering properties (Irmak and Dunford 2005). In a later study the effect of genotype and environment on the bioactive compounds in three wheat varieties, Jagger, Trego, and Intrada, were examined (Chen and others 2009a; Chen and others 2009b). Trego had the highest PC and phytosterol (PS) content followed by Intrada. The antioxidant properties of Trego wheat have been reported (Zhou and Yu 2004a). Therefore, Intrada variety was chosen for this study.

Intrada is a hard white winter wheat developed cooperatively by the Oklahoma Agricultural Experiment Station (OAES), Kansas Agricultural Experiment Station and United State Department of Agricultural Research Service (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'. This variety has a high yield and test weight and potential for its end-use value in domestic and export bread markets. Intrada grows well in the southern High Plains and performs best under dryland production conditions in western Oklahoma and in neighboring states (Carver and others 2003).

2.2 WHEAT BIOACTIVE COMPOUNDS

Wheat grain contains a number of bioactive compounds, including tocopherols and tocotrienols which are also referred to as tocols in this dissertation, carotenoids, phenolic acids, organic acids, PC and PS. These bioactive compounds play important structural and defense roles in wheat grain and contribute to the nutritional quality of the grain.

2.2.1 TOCPHEROLS AND TOCOTRIENOLS

Tocopherols and tocotrienols are fat soluble compounds. Each group has four isomers: α, β, γ, δ (Okarter and Liu 2010). Vitamin E is the generic term for all eight tocopherol and tocotrienol isomers (Eitenmiller and Lee 2004). Both tocopherols and tocotrienols have a chromanol ring and an aliphatic side chain in their chemical structure. The difference between tocopherol and tocotrienol is that the side chain of tocopherol is saturated while the side chain of tocotrienol is unsaturated (Miyazawa and others 2009; Theriault and others 1999). Tocols are only found in photosynthetic bacteria, algae, plants and animals (MunnéBosch 2007). Vegetable oil and cereal grains such as barley, oats, wheat, rye and rice are excellent sources of tocols (Tiwari and Cummins 2009). Palm oil is one of the most abundant sources of tocotrienols. Crude palm oil contains up to 800 mg / kg tocotrienols and most of these are α-tocotrienol and γ-tocotrienol (Sen and others 2007a).

Tocols can effectively react with lipid peroxyl radicals to form a relatively stable tocopheroxyl radical and lipid hydroperoxide and stop the oxidation chain reaction (Sies and Stahl 1995; Blokhina and others 2003; Burton and Traber 1990). Because of their strong antioxidant ability, they can reduce inflammation and angiogenesis (Wells and others 2010). The antioxidant activities of all eight tocols isomers are not the same due to the methylation pattern and the amount of methyl groups on the chromanol ring. The order of the strength of antioxidant activity of tocopherols is as follows: $\alpha > \beta > \delta > \gamma$ (Blokhina and others 2003). α-Tocopherol is the most potent natural antioxidant to scavenge lipid peroxyl radicals and reactive oxygen and nitrogen species. It has been reported that α-tocopherol is able to protect lipid oxidation and prevent chronic diseases

such as atherosclerosis, cancer and Alzheimer's disease (Tucker and Townsend 2005; Schneider 2005; Paradiso and others 2009; Ni and Yeh 2007; Traber and Atkinson 2007). There are studies indicating that γ-tocopherol possesses anti-inflammatory properties and has a better ability to trap reactive nitrogen species than α-tocopherol (Reiter and others 2007). The Food and Nutrition Board recommends that the intake of α-tocopherol should be limited to 1000 mg per day (Medicine 2000).

In several studies tocotrienols have been shown to be stronger antioxidants than tocopherols (Choi and Lee 2009; Qureshi and others 1997; Das and others 2007). Das and others (2007) clearly demonstrated that tocotrienols, especially α- and γ-tocotrienols, provide protection against cardiovascular disease by lowering serum cholesterol levels and reducing oxidative reactions. Sen and others (2007a; 2007b; 2006) extensively reviewed the health benefits of tocotrienols and concluded that tocopherol and tocotrienol isomers have both common yet some unique biological functions. Tocotrienols are the important part of the vitamin E family, as they possess potent antioxidant, anticancer and neuroprotective activity and cholesterol lowering properties. It has been shown that tocotrienols, especially δ-tocotrienol, remarkably suppresses liver and lung carcinogenesis in mice (Wada and others 2005). Miyazawa and others (2009) pointed out that the anticancer ability of tocotrienols was related to their antiangiogenic activity. The safe dose of various tocotrienols for human consumption was recommended as 200-1000 mg / day (Yu and others 2006).

Wheat is a good source of tocols. In einkorn wheat, the total tocols content was about 77.96 μg / g, with β-tocotrienol being the most abundant at 48.22 μg / g, , followed by α-tocotrienol at 12.77 μg / g , α-tocopherol at 12.18 μg / g and β-tocopherol at 4.79 μg

/ g. Einkorn wheat contained significantly higher tocols content than other wheat varieties (Hidalgo and others 2006). Zhou and others (2005) examined the tocols content in two wheat varieties from two locations and detected α-tocopherol, δ-tocopherol and γ-tocopherol. α-Tocopherol and γ-tocopherol were the two major tocols in the samples. In a study of eight soft red wheat genotypes grown in Maryland, α-tocopherol was reported to vary from 3.4 to 10.1 μg / g (Moore and others 2005). The studies discussed above indicate that content and compositions of tocols in wheat are affected by genotype and environment.

Tocols are not evenly distributed in the wheat kernel. They are concentrated in the germ and outer layers of the grain, with very small amount of tocols present in the endosperm. It has been reported that total tocols content in wheat bran was almost two-fold higher than that of whole wheat flour (Khan and Shewry 2009). Wheat germ contains specifically high contents of α-tocopherol and β-tocopherol. About 256 μg / g of α-tocopherol and 114 μg / g of β-tocopherol were found in wheat germ respectively (Morrison and others 1982). Similar results were reported by Piironen and others (1986). Wheat germ oil (WGO) is rich in tocopherols. α-Tocopherol, β-tocopherol and γ-tocopherol were present in four commercial WGO samples (Eisenmenger and Dunford 2008). Samples obtained by supercritical CO_2 extraction had significantly higher tocopherol content (26.86 mg / g oil) than the rest of the three commercial oils (15.08 mg / g oil, 7.7 mg / g oil, and 7.97 mg / g oil). α-Tocopherol consisted 90 % of the total tocopherols.

2.2.2 PHENOLIC ACIDS

Phenolic acids are widely distributed in plants (Schieber and others 2001; Häkkinen and others 1999). The chemical structure of a phenolic acid comprises of an aromatic ring to which one or more hydroxyl groups are attached. Phenolic acids can be categorized into two groups according to their chemical structures. These two groups are referred to as hydroxycinnamic and hydroxybenzoic acids which have similar chemical structures but are distinguished by the numbers and positions of the hydroxyl groups on the chromatic ring (Khan and Shewry 2009). Hydrocinnamic acids include *p*-coumaric, caffeic, ferulic, and sinapic acids, while hydrobenzoic acids are *p*-hydroxybenzoic, protocatechuic, vannilic, syringic, and gallic acids (Liu 2007).

Phenolic acids are of great interest because they possess antioxidant properties and provide protection against chronic diseases such as cancer (Yi and others 2005) and cardiovascular diseases (Morton and others 2000). The antioxidant ability of phenolic acids is related to the hydroxyl substitute on the aromatic ring. The position and number of the hydroxyl substitutes affect the hydrogen atom donating ability of phenolic acids which further influence their radical scavenging ability. Therefore, different phenolic acids have varying antioxidant properties (Robbins 2003). When functioning as free radical scavengers, phenolic acids donate a proton to the radical to terminate free radical chain reactions. Hydroxycinnamic acids have a stronger radical scavenging ability than the hydroxybenzoic acids. Addition of the hydroxyl and methoxyl substituents on the aromatic ring could improve the total antioxidant properties of phenolic acids (Yu 2008).

Ferulic, vanillic, caffeic, syringic, hydroxybenzoic, and *p*-coumaric acid are commonly found in wheat (Okarter and others 2010; Kim and others 2006). Ferulic acid

is the major cinnamic acid in cereals, and is the most abundant phenolic acid in wheat grain. Ferulic acid is concentrated in the aleurone, pericarp, and embryo cell walls of various cereals. It is present in the endosperm at low concentration (Smith and Hartley 1983; Khan and Shewry 2009). Among the eleven wheat varieties evaluated, Cham1 had the highest ferulic acid content, up to 588.37 μg / g of grain (Adom and others 2003). Another study carried out on eight wheat varieties grown in Maryland showed that total ferulic acid content of the samples was higher than the values reported earlier, 456-621 μg / g grain. Vanilic, syringic and *p*-coumaric acids were also found in the samples but in lesser amounts than ferulic acid (Moore and others 2005). Ferulic acid was concentrated in the aleurone layer, 7.22-9.06 μg / mg (Antoine and others 2003). The total phenolic acids content in wheat bran was 4527 μg / g which was significantly higher than that of whole wheat flour (1342 μg / g) (Mattila and others 2005). The ferulic acid content of bran and germ was 52-70 folds higher than the endosperm (Adom and others 2005).

2.2.3 CAROTENOIDS

Carotenoids are a large group of pigmented compounds that are yellow, orange and red in color. Currently, more than 600 carotenoids have been identified in plants and microorganisms. About 50 of these carotenoids have vitamin A activity (Liu 2007; Mayne 1996). Carotenoids can only be synthesized by plants and microorganisms, but not by animals, so humans and animals get carotenoids from dietary sources (Paiva and Russell 1999). The carotenoids share a common molecular structure which consists of a polyisoprenoid and a long hydrocarbon chain with conjugated double bonds (Cadenas and Packer 2002). Carotenoids are classified into hydrocarbons (carotenes, like β-

carotene) and their oxygenated derivatives (xanthophylls such as lutein and zeaxanthin) (Marquart and others 2007). Carotenoids are essential for photosynthesis, reproduction and photoprotection. They are also the major dietary source of retinol for humans. Furthermore, carotenoids are lipid soluble antioxidants which can effectively quench the electronically excited molecules, particularly the singlet molecular oxygen (Krinsky 1989; Britton 1995; Di Mascio and others 1991). Both the number of conjugated double bonds and the end groups influence the quenching ability of carotenoids (Paiva and Russell 1999). Carotenoids can interact with free radicals in three ways: electron transfer, hydrogen donation and the formation of a radical species. There are various types of reactive oxygen species in human body. Chemical characteristics of these species strongly affect the mechanisms and rate that carotenoids can quench free radicals. For instance, β-carotene is very reactive to peroxyl radicals but inactive to $O_2^{\cdot -}$ (Young and Lowe 2001).

Many foods are rich in carotenoids. These are mainly colored fruits and vegetables, examples include carrots, tomatoes, watermelon and pumpkins (Krinsky 1989; Holden and others 1999). Some carotenoids, such as α-carotene, β-carotene, and β-cryptoxanthin have provitamin A activity, which means they can be enzymatically converted to retinol necessary for vision (Voutilainen and others 2006; Krinsky 1989). Carotenoids can protect lipid membranes from peroxidation and LDL containing lipids from oxidation (Bendich and Olson 1989). Therefore, the consumption of carotenoids can be credited with reducing the risk of cancers, providing protection against DNA damage, preventing cardiovascular diseases, enhancing the immune system, and providing protection against age related macular degeneration and cataract (Bendich 1989; Young

and Lowe 2001; Ziegler 1989; Mayne 1996; McNulty and others 2008; Nishino and others 2009; Krinsky 1989). However, there are reports indicating that β-carotene may increase the risk of cardiovascular disease and lung cancer in smokers (Mayne 1996).

Even though the carotenoid content in wheat is not very high, wheat could provide significant amounts of carotenoids as wheat is readily found in most daily diets. β-Carotene, zeaxanthin, cryptoxanthin and lutein are the major carotenoids found in wheat, while lutein is the primary xanthophyll (Khan and Shewry 2009). Moore and others (2005) reported that total carotenoids contents in eight soft wheat varieties grown in Maryland ranged from 1.30 to 1.68 µg / g and lutein was the most abundant carotenoid at 0.82-1.14 µg / g followed by zeaxanthin and β-carotene. In another study of hard red winter wheat, lutein was also found to be the predominant carotenoid ranging from 0.97 to 2.43 µg / g. Significant amounts of zeaxanthin, β-cryptoxanthin and β-carotene were detected in wheat samples as well (Zhou and others 2005). Adom and others (2005) compared carotenoid contents in two wheat fractions, one was a bran and germ mixture and the second one was an endosperm sample. Carotenoid content of bran and germ mixture was almost two-fold higher than that of the endosperm sample.

2.2.4. ORGANIC ACIDS

Organic acids are low molecular weight compounds that are widely present in nature and contain one or more carboxyl groups in their chemical structure (Jones 1998). Wheat organic acids are related to Aluminum (Al) tolerance. The harmless Al is converted to the toxic form, $[Al(H_2O)_6]^{3+}$ in acidic soil adversely affecting plant growth and production (Darkó and others 2004). It has been suggested that the secretion of

organic acids from plant roots could contribute to the detoxication of Al. Organic acids can chelate ionic Al to form a stable compound to prevent binding of Al with plant roots (Li and others 2000; Delhaize and others 1993). The studies on organic acids in wheat extracts are limited. Nelson and Hasselbring (1931) reported that major organic acids in wheat were malic, aconitic and citric acids. Burke and others (1985) studied organic acids in seven wheat varieties by using a capillary gas chromatography method. In the latter study, both two and four weeks old wheat grain samples were examined. Succinic, malic, aconitic and citric acids were detected in the two weeks old wheat while no succinic acid was detected in the four week old wheat.

Organic acids are widely used in food industry to provide color and acidic flavor. Citric acid and ascorbic acid are commonly used to stabilize fats and oils. Some organic acids can chelate metals to enhance the activity of other antioxidants (Pokorný 1991; Asami and others 2003).

Many antioxidants may work synergistically with other antioxidants to provide protection against reactive oxygen radicals. Truscott (1996) investigated the interaction of β-carotene, α-tocopherol and vitamin C and found that β-carotene could repair vitamin E radical and similarly vitamin C could repair the β-carotene radical. β-Carotene together with α-tocopherol can scavenge various radicals to inhibit a chain reaction (Stahl and others 1998).

2.2.5 POLICOSANOL

PC are a mixture of high molecular weight (20-36 carbon) aliphatic primary long-chain alcohols. Docosanol (C22), tricosanol (C23), tetracosanol (C24), hexacosanol

(C26), octacosanol (C28), and triacontanol (C30) are the main PC in wheat varieties (Irmak and Dunford 2005; Irmak and others 2006; Chen and others 2009b). PC are able to lower total cholesterol and LDL and increase high density lipoprotein levels in serum (Aneiros and others 1995; Gouni-Berthold and Berthold 2002; Brufau and others 2008). The studies of PC as a lipid lowering agent are extensive (Mas and others 1999; Castano and others 1995; Hernandez and others 1992; Varady and others 2003). Studies carried out on 85 patients for 3 or more years indicated that PC at a dose of up to 20 mg / day was safe and well tolerated (Gouni-Berthold and Berthold 2002). It was postulated that PC reduced the risk of coronary heart disease (CHD) by inhibiting platelet aggregation and proliferation (Gouni-Berthold and Berthold 2002; Borg 1991). Although many studies demonstrated the efficacy of PC to control serum LDL levels, there are also reports contradicting the latter studies (Varady and others 2003).

Irmak and Dunford (2005) examined the distribution of PC in wheat fractions and reported that PC were concentrated in bran and germ fractions of wheat grain. A recent study showed that wheat straw had a higher PC content than wheat germ and bran fractions (Dunford and Edwards 2010). The PC content and compositions of 31 wheat varieties grown in Oklahoma were evaluated (Irmak and Dunford 2005). The study demonstrated that Trego and Intrada had the highest PC content among the varieties studied. Tetracosanol, hexacosanol and octacosanol were the major PC components in all varieties. Both genotype and environment had significant effects on PC content and compositions in wheat (Irmak and others 2007; Chen and others 2009b).

2.2.6 PHYTOSTEROL

PS, also referred to as plant sterols, are isoprenoid compounds. They are cholesterol-like compounds containing a four-ring steroid nucleus (Ostlund 2002; Khan and Shewry 2009). PS differ in chemical structure from cholesterol by having a side chain of an ethyl or methyl group (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 which are present in smaller amounts than other PS (Micallef and Garg 2009; Liu and others 2007). Sitosterol, stigmasterol and campesterol are the most abundant PS in wheat (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). So far, more than 250 PS have been identified in plants or marine animals (Brufau and others 2008).

Petersons (1951) was the first to report the serum cholesterol lowering properties of PS. The hypocholesteremic properties of PS have been studied extensively (Miettinen and others 2000; Jones and Ntanios 1998; Brufau and others 2008). A meta-analysis of 41 clinical trials indicated that the intake of 2 g of PS per day could reduce LDL cholesterol level by 10 % (Katan and others 2003). A number of clinical studies demonstrated that PS can lower LDL levels by as much as 10 -14 % in normal and hypercholesteromic adult males and females (Kritchevsky and Chen 2005; Plat and Mensink 2005). Another clinical study confirmed that patients already taking statin drugs could reduce their LDL levels an additional 10 % by inclusion of PS in their diet (Blair and others 2000). The intake of PS up to 3 g per day has been considered safe (Marangoni and Poli 2010).

Furthermore, PS can provide protection against certain types of cancer such as colon, breast and prostate cancers (Awad and Fink 2000; Tapiero and others 2003; Ifere and others 2009). Some studies linked the health benefit of PS to their antioxidant activity (Vivancos and Moreno 2005). Yasukazu and Etsuo (2003) reported that PS prevent peroxidation of vegetable oil by functioning as an antioxidant to scavenge free radicals.

PS are naturally found in vegetables and seed oils, but also in pulses and dried fruits (Quilez and others 2003). Dietary sources of PS include nuts, grains, beans and plant oils (Ling and Jones 1995; Marangoni and Poli 2010). In cereal grains, PS are mostly found in bran and are extractable as part of bran oil and waxes (Wu and others 2007). Hence whole grains are better sources of PS than refined flour (Piironen and others 2000). Due to the health benefits of PS, food industry has already produced products enriched in PS. For example PS enriched margarines, yogurts, salad dressings, milk and snack bars are available in the market today. These PS enriched foods can be an effective way to reduce the total cholesterol and LDL cholesterol levels (Micallef and Garg 2009).

Several groups have examined the PS content and composition of wheat grain fractions and wheat germ oil (Eisenmenger and Dunford 2008; Jiang and Wang 2005; Hakala and others 2002; Nystrom and others 2007). Wheat germ has a much higher PS content (2.4 mg / g) (Jiang and Wang 2005) than bran (1.2 mg / g) and whole wheat (1.8 mg / g) (Piironen and others 2000). Compared to other commercial oils, WGO has extremely high PS content, with sitosterol and campesterol being the major PS (Itoh and others 1973; Anderson and others 1926). Eisenmenger and Dunford (2008) showed that wheat milling, WGO extraction and oil refining techniques had significant effects on the

PS contents of the final product. Also genotype and environment had significant effects on PS contents and compositions of wheat (Chen and others 2009a; Dunford and Edwards 2010).

2.3 WHEAT HEALTH BENEFITS

Grains account for about two thirds of the energy and protein intake in developing countries (McKevith 2004). In the U.S., approximately a quarter of the calories consumed are from grain products (Liu 2007). In addition to providing energy, whole grains are rich in fiber, protein, minerals, vitamins and bioactive compounds (Liu 2007; Okarter and Liu 2010). Some of these bioactive compounds have antioxidant functions. Due to its high nutritional value, USDA recommends daily consumption of 3 oz of whole grain products which include bread, pasta and crackers (USDA 2010d).

Numerous studies have demonstrated that intake of whole grains and whole grain products can reduce the risk of cardiovascular disease (Jacobs and others 1998; Liu and others 1999; Liu 2002; Hu and Willett 2002; Liu and others 2000b), diabetes (Meyer and others 2000; Liese and others 2003) and some cancers (Slavin 2000; Ferguson and Harris 1999; Adlercreutz 2002; McCullough and others 2003). In the Nurse's Health Study, Liu and others (2000a) found an inverse relationship between whole grain intake and risk of type 2diabetes. About 76,000 female nurses were part of this 10 year research project. The female nurses who consumed approximately 21 servings of whole grains per week had a 27 % lower risk of diabetes than nurses who consumed less than one serving per week. Rimm and others (1996) examined the association between vegetable, fruit, plus cereal intake and a risk for myocardial infarction (MI) in 43,757 U.S. health professionals,

aged 40 to 75. Cereal fiber was most strongly associated with reduced risk of coronary heart disease (CHD).

It has been shown that WGO 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). In addition, WGO feeding is shown to be effective in improving both fertility and successful delivery of golden hamsters (Soderwall and Smith 1962). The U.S. Food and Drug Administration (FDA) approved the health claim notification on food packages containing at least 51 % (w / w) whole grain products. The claim states that the consumption of whole grain foods may lower the occurrence of CHD and cancers (FDA 1999). Intake of whole grain foods is also recommended by the American Heart Association (2010), the American Diabetes Association (2010), and the American Cancer Society (2010).

Researchers have attributed health benefits of wheat to the bioactive wheat components with antioxidant properties (Baublis and others 2000; Liyana-Pathirana and Shahidi 2007b; Liu 2007). Miller and others (2000) pointed out that the antioxidant content of whole grain foods was comparable to fruits and vegetables. A recent study showed that antioxidant capacity of bran was more than two times higher than that of the refined flour (Yu 2008). Similarly, Liyana-Pathirana and Shahidi (2007a) reported that bran had higher antioxidant capacity than flour and shorts. Zhou and others (2004a) compared the antioxidant properties of Swiss red wheat grain fractions and found that bran had higher antioxidant capacity and more phenolic acids than whole grain.

Phytochemical contents and antioxidant activity of milling fractions from five different varieties (CaledoniaNY, CaledoniaMI/OH, CayugaNY, RoaneNY, RoaneMI/OH) of wheat grain were evaluated by Adom and others (2005). It was found that bran and germ fractions had higher antioxidant activity and phytochemical contents than the endosperm fraction of all five varieties examined. Liyana-Pathirana and others (2006) reported that antioxidant capacity of pearled wheat decreased when grain outer layers were removed. Moreover, the resultant byproducts from pearling possessed higher antioxidant capacity than the respective pearled wheat regardless of wheat class. Beta and others (2005) also observed that pearling was an effective method to enrich phenolic antioxidants in wheat bran fraction. Aleurone is an important part of wheat grain. Even though studies about aleurone are scarce, the limited data available in the literature indicates that aleurone is a good source of folate (Fenech and others 1999), and has a higher content of ferulic acid than bran and flour fractions (Mateo Anson and others 2008). Aleurone extracts have a higher free radical scavenging ability than those of bran and whole grain (Zhou and others 2004a).

2.4 ANTIOXIDANTS

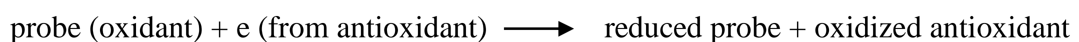
Free radicals have been proven to cause oxidative damage to lipids, proteins and nucleic acids, which lead to chronic diseases (Devasagayam and others 2004; Machlin and Bendich 1987). A free radical is a molecule with at least one unpaired electron in the outer orbit (Clarkson and Thompson 2000). Because of this unstable structure, free radicals are highly reactive and they can further convert into other intermediates, for examples peroxyl radical, alkoxyl radical (Mesa and others 1994), and nitrogen dioxide

(Fang and others 2002). Antioxidants are effective at low concentrations to retard or inhibit oxidation of a free radical. Antioxidants can counteract and eliminate the negative effects of free radicals and retard or inhibit oxidation; thereby reducing blood pressure and the risk of CHD and suppressing tumor formation (Young and Woodside 2001; Moon and Shibamoto 2009). Additionally, antioxidants play an important role in the food formulations as they prevent lipid oxidation which causes rancidity, deterioration of color, flavor, texture, nutritional quality and the safety of foods (Lanari and others 2004; Arabshahi-D and others 2007).

There are three steps involved in an oxidation reaction: initiation, propagation and termination. During the initiation step, a lipid molecule reacts with reactive oxygen species and generates a highly reactive allyl radical. In the propagation stage, a chain reaction starts. The unstable allyl radical can further react with molecular oxygen to form a lipid peroxyl radical which is also an unstable species. This lipid peroxyl radical can readily react with lipid molecules to form lipid hydroperoxides and alkoxyl radicals. The radical formation reaction stops when two radicals react and produce a non-radical species. Antioxidants can react with the free radicals generated during lipid oxidation resulting in retardation or inhibition of oxidation reactions. The antioxidant free radical generated in the reaction could react with the lipid free radical to produce peroxyl antioxidant compounds. So, based on the above reactions, antioxidants can be classified into two groups: primary or chain breaking and secondary or preventative. Primary antioxidants mainly delay or stop the initiation step or inhibit the propagation step. Secondary antioxidants reduce the oxidation rate (Antolovich and others 2002).

There are a variety of compounds naturally present in plant and animal tissues that possess antioxidant properties. These antioxidants may react with various free radicals in different ways. Hence there is no single assay that can accurately reflect all free radical and antioxidant reactions taking place in a complex system due to the multiple reaction mechanisms involved. Thus, several methods have been developed to evaluate total antioxidant capacity of a sample.

Based on the chemical reactions, antioxidant assays can be divided as follows: the hydrogen atom transfer (HAT) or the single electron transfer (ET) based assays. The principle of HAT based methods is the formation of delocalized stable radicals which slow down or inhibit the chain reaction. This type of assay involves three components: an antioxidant (AH), a free radical and a substrate. The free radical can react with both AH and the substrate. During the reaction, AH donates its hydrogen atom to the free radical faster than the free radical reacts with the substrate thus retarding the oxidation of the substrate. Most HAT assays follow competitive reaction kinetics. Such assays include the oxygen radical absorbance capacity (ORAC), the total radical trapping antioxidant parameter (TRAP), and the inhibition of LDL oxidation. ET based assays have only two components, antioxidants and oxidant, in which the oxidant is also used as an indicator to monitor the reaction. A redox reaction takes place during the process;



The oxidant is the probe which abstracts an electron from the antioxidant, resulting in a color change of the probe. The degree of the color change is proportional to the antioxidant concentration. This type of assays include Total Phenolic Content (TPC) assay using Folin-Ciocalteu Reagent and 2,2-Diphenyl-1-Picrylhydrazyl radical

scavenging capacity assay (DPPH). (Huang and others 2005; Prior and others 2005; Alamed and others 2009; MacDonald-Wicks and others 2006).

2.4.1 Oxygen radical absorbance capacity

ORAC assay is largely used to evaluate the antioxidant capacity of both botanical and animal tissue extracts (Prior and Cao 2000). This method uses competitive kinetics to assess the ability of antioxidants to compete with fluorescein (FL), a molecular probe, to scavenge the peroxyl radicals generated by 2, 2′-Azodi (2-amidinopropane) dihydrochloride (AAPH) (Huang and others 2005). The generated peroxyl radical can oxidize FL to form a non-fluorescent product. Added antioxidant can react with the formed peroxyl radical by donating a hydrogen atom to prevent the oxidation of the FL (Ou and others 2001; Huang and others 2002; Prior and others 2005). Trolox, a vitamin E analogue, is used as a standard in this method. Phosphate buffer is used as blank. Antioxidant, phosphate buffer and Trolox are mixed with fluorescein solution and AAPH. The mixture is incubated at 37 °C. The fluorescence intensity [485 nm (excitation)/ 535 nm (emission)] is measured every half minute for 45 min. During the reaction, the intensity of FL is decreased and the loss of FL is recorded by a fluorometer (Fauconneau and others 1997). A typical ORAC assay kinetic curve is shown in Figure 1. Antioxidant capacity is calculated by using the area under the kinetic curve (AUC) and net AUC (AUC sample-AUC blank) (Fauconneau and others 1997). The ORAC value in μmoles trolox equivalent (TE) / g sample is calculated as:

[(AUC sample - AUC blank) / (AUC Trolox - AUC blank)] × (μmoles Trolox / g sample). ORAC value is expressed as μmoles (TE) / g sample. This method simulates the reaction

of peroxyl radicals and antioxidants in both food and physiological systems, and it is applied to both hydrophilic and lipophilic systems (Bisby and others 2008; Sánchez-Moreno 2002). ORAC assay reflects the quenching ability of antioxidants to the peroxyl radicals, the higher the value, the stronger the antioxidant capacity is.

2.4.2 2, 2-Diphenyl-1-Picrylhydrazyl radical scavenging capacity assay

The DPPH method was introduced by Blois in 1958 based on the measurement of the reducing power of antioxidants toward DPPH free radical (Blois 1958). DPPH is astable and commercially available organic nitrogen radical. By virtue of the delocalization of the spare electron, it has a deep violet color and a maximum UV-Vis absorption at 517 nm. This assay proceeds via both electron and hydrogen atom transfer reactions (Prior and others 2005). The reaction rate is determined by following the rate of electron transfer from the antioxidant to the DPPH radical. The rate of next reaction step, hydrogen transfer from the antioxidant to the DPPH radical, is not significant (Foti and others 2004). Thus, this reaction is considered as ET based assay (Huang and others 2005).

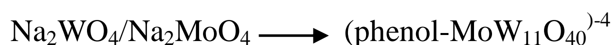
Briefly, DPPH in methanol is mixed with the antioxidant sample and allowed to react. For this assay different initial DPPH concentrations and reaction times have been reported in the literature (Iqbal and others 2007; Yu and others 2003; Zhou and others 2004b). The absorbance of the reaction mixture is monitored at 517 nm. As the reaction proceeds the color fades from purple to yellow because of the reduction of DPPH radical and the absorbance decreases as well. The percentage inhibition of the DPPH radical is calculated as:

$$\% \text{ DPPH}_{\text{inhibition}} = (\text{Abs}_{\text{ctrl}} - \text{Abs}_{\text{test}}) / \text{Abs}_{\text{ctrl}} \times 100$$

As a stable nitrogen radical, DPPH radical differs from the highly reactive and transient peroxyl radicals in lipid oxidation. For instance, some antioxidants react immediately with peroxyl radicals. However, the same antioxidants may react slowly or even be inert to DPPH radical (Prior and others 2005). This assay is easy, extremely sensitive and accurate, hence, it is commonly used to evaluate antioxidant capacity of natural materials (Singleton and others 1999; Sánchez-Moreno 2002), especially the antioxidant ability of polyphenolic compounds (Kikuzaki and others 2002; Villaño and others 2007).

2.4.3 Total phenolic content assay by Folin-Ciocalteu Reagent

Phenolics such as phenolic acids are strong antioxidants. The total phenolic content of a sample can be used as an estimate of its antioxidant capacity. Folin-Ciocalteu Reagent (FCR) is a mixture of tungsten and molybdenum oxides. The oxidation of phenols by the metals in this solution yields a blue colored product with the maximum absorption at 765 nm. The intensity of light absorption is proportional to the concentration of phenols (Huang and others 2005). This method is simple, precise and sensitive. Because the reaction proceeds slowly under acidic conditions, the pH has to be adjusted to basic conditions by using sodium carbonate solution (Prior and others 2005). Dissociation of the phenolic proton leads to a phenolate anion, which is able to reduce FCR. In fact, this method actually measures the reducing ability of the sample and it is an ET based assay (Huang and others 2005).



$$Na_2WO_4/Na_2MoO_4 \longrightarrow (\text{phenol-}MoW_{11}O_{40})^{-4}$$

$$Mo\ (VI)(yellow) + e^{-} \longrightarrow Mo\ (V)(blue)$$

The TPC method can be described as follows: mixing the FCR, antioxidant sample, sodium carbonate solution and deionized (DI) water and incubating the mixture at room temperature for 2 h. Absorbance of the mixture at the end of 2 h is recorded at 765 nm. A standard curve which is prepared by plotting the absorbance values of gallic acid standard solutions against their concentrations is used for calculation. TPC value is calculated as: mg gallic acid equivalent (GE) / g sample = GE mg / mL × sample volume mL / sample g

2.5 WHEAT MILLING

2.5.1 WHEAT GRAIN STRUCTURE

A wheat kernel is a single-seeded fruit which consists of three parts: endosperm, bran and germ (Figure 2) (Posner and Hibbs 2005; Yu 2008). Endosperm is used as the energy source during seed germination and early plant development (Yu 2008). Endosperm is the major part of the wheat kernel, which takes up about 82 % of the kernel mass. The main components of endosperm are starch and protein (Yu 2008; Khan and Shewry 2009). Bran is the outer layer of the kernel and a byproduct of grain milling. Its function is to protect the grain against mechanical and insect damage. Wheat bran comprises approximately 15 % of the kernel weight. Bran has several layers, epidermis, hypodermis, intermediate-cell, cross-cell, tube-cell, and testa (Posner and Hibbs 2005). Carbohydrates are the primary components in wheat bran, followed by protein, ash and lipids. Besides those, bran also contains some vitamins and phytochemicals (Yu 2008). Aleurone is the outermost cell layer in the endosperm. However, aleurone normally

remains attached to the bran and is removed with bran during conventional milling (Yu 2008; Posner and Hibbs 2005). Aleurone layer makes up to 45-50 % of the bran fraction and is rich in proteins and enzymes which are important for wheat germination (Šramková and others 2009; Buri and others 2004). Germ is located at the base of the kernel (Khan and Shewry 2009). Germ comprises two major components: embryonic axis which is composed of the primary root and shoot, and scutellum. These two parts are separated from endosperm by the epithelial layer. Germ plays an important role in germination. The embryonic axis grows into the seedling and scutellum provides the nutrition to the embryonic axis (Posner and Hibbs 2005; Yu 2008). The germ is about 2-3 % of the kernel weight. It contains abundant protein, lipids, and minerals (Šramková and others 2009). Additionally, wheat germ is a good source of vitamins and phytochemicals (Yu 2008).

2.5.2 WHEAT MILLING

Wheat grain must be processed before it can be used as human food. Milling process separates bran, germ and endosperm. The separation is achieved through three steps：cleaning, tempering, and milling. Wheat always arrives in the mill with impurity materials such as strings, straw, wood, stones or metal. They must be removed to prevent damage to the wheat quality and the milling equipment. This foreign matter is removed in the cleaning system. Cleaning removes undesirable components by classifying incoming materials by size, shape, density, and magnetism (Posner and Hibbs 2005). The dry milling process at very low grain moisture content is not efficient for separating endosperm, bran and germ. Thus, it is necessary to condition grain prior to milling. The

conditioning process involves two steps: adding water and then tempering for sufficient time to allow moisture to penetrate the kernels. The optimal tempering will bring the moisture content of the grain to 13.5 - 15.0 % (w/w). Tempering normally takes 6-10 h. By conditioning wheat, the endosperm becomes malleable allowing separation from bran and germ with less force. With sufficient water, bran is toughened and will not pulverize during milling (Khan and Shewry 2009; Dendy and Dobraszczyk 2001).

The traditional milling system includes the following unit operations: breaking, size reduction, tailing, and sieving. The break system consists of pairs of rolls which rotate in opposite directions to breakdown the wheat kernels (Figure 3). Size reduction systems are made of smooth or corrugated rolls that reduce endosperm into fine particles. The objective of the tailing system is to recover endosperm by reducing particle size small enough to pass the sieves to get a better separation. The ground material is then fractionated by a sieving system that contains several sieves of different sizes (Atwell 2001; Posner and Hibbs 2005; Yu 2008).

Due to the limitations of the roller mills, complete separation of endosperm from bran fraction is not easy to achieve. Debranning process was developed as a pretreatment to be used prior to milling to improve flour yield and prevent bran carryover to the refined flour. Debranning, also called pearling, is a process that successively removes bran layers by friction and abrasion (Dexter and Wood 1996). A cross sectional image of a debranner is shown in Figure 3. Prior to debranning, wheat samples are conditioned with water and tempered for a short time so that the water is distributed only in the seed coat. After preconditioning, bran layers are removed sequentially in equipment containing a rotating abrasive stone. During the debranning process a rotating vane

hollow shaft leads kernels to the abrasive stone facilitating kernels to rub against each other and the abrasive stone surface (Liu and others 2008; Bottega and others 2009). Air introduced into the debranner helps to separate fine bran from the residual grain (Dexter and Wood 1996).

The purpose of the traditional wheat milling process is to produce refined wheat flour. Wheat bioactive compounds end up in the milling by-products which are mostly used in low value applications such as animal feed. Hence, wheat bran and germ are excellent sources for recovery of health beneficial bioactive compounds and value-added product development. A fundamental understanding of the distribution of valuable components in wheat fractions is critical for successful process development and optimization.

2.5.3 TANGENTIAL ABRASIVE DEHULLING DEVICE

A clean separation of germ and bran from endosperm is necessary to ensure the maximum recovery of desirable bioactive compounds. Tangential Abrasive Dehulling Device (TADD) can be used to achieve this goal. The concept of tangential abrasion which is similar to pearling was brought forth in 1960s by Hogan and others (1964) who found that the outer layers of rice kernels could be successively removed by tangential abrasion without damaging the kernels. The original TADD was developed in 1981 by Oomah and others (1981), which was able to process eight samples simultaneously. This first prototype TADD has been used to evaluate dehulling characteristics of barley and sorghum by measuring abrasion rate, flour extraction rate and dehulling reproducibility. It was found that TADD was an efficient instrument to examine dehulling properties of

grain and generated reproducible results. The instrument was easy to operate and did not take much space. Reichert and others (1986) further developed this instrument allowing up to 12 samples to be dehulled at a time.

TADD has been extensively used to evaluate the hardness of sorghum (Lawton and Faubion 1989), barley (Oomah and others 1981), and wheat (Reichert and others 1986). Dehulling characteristics of flaxseed (Oomah and Mazza 1997; Oomah and others 1996) , sorghum (Lochte-Watson and others 2000), and chickpea (Singh and others 1992) have also provided valuable data. A recent study illustrated that TADD can be used to enrich phytochemicals in sorghum milling fractions (Awika and others 2005). This study used TADD to successively remove sorghum bran layers at 1 min intervals. Six fractions were collected. Phytochemical content and antioxidant activity of the fractions were examined. The bran factions collected at 1 and 2 min showed the highest phenols content and antioxidant capacity, which was about 3 to 6 times higher than that of the whole grain sample. To date, no study has been reported on the efficiency of TADD to recover the antioxidants from wheat grain.

CHAPTER 3

MATERIALS AND METHODS

3.1 SAMPLE SELECTION

Intrada wheat was collected from Oklahoma State University (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), Oklahoma. Alva and Balko are both on farm fields, and the Goodwell location is on the OSU research station. Plots had eight 15 cm wide and 12 m long rows. Seeds 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 dryland: 10-01-04, Balko: 10-18-04, Alva: 09-30-04. All fields were managed under "grain only" practices. Whole wheat grain samples were collected after normal harvest. All the Intrada samples from different locations and different agricultural practices were mixed prior to the experiments. A commercial aleurone sample from Cargill Co. (MN, U.S.A.) was included as a reference. Samples were stored in paper bags and kept in a freezer at -20 °C after being received in our laboratory until the testing began.

3.2 MILLING

Cleaned whole Intrada wheat grain samples were tempered to 15 % moisture content overnight using a tempering instrument (GE A-G Gear Motor, MOD: 14PCP4265, Ford Wayne, IN). After tempering, the grain sample was milled using a quadrumat senior mill (C.W. Brabender Instrument, INC, NJ). A sieve tester (Gilson Company, Inc, Ohio) was used to separate bran from endosperm after milling. 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. Fraction with particle size over 500 μm has been referred to as "bran" in this study. Fractions with particle size at 150- 500 μm and smaller than 150 μm were break flour and shorts respectively. Only the bran fraction which was referred to as "bran" throughout this dissertation was used in the further analyses.

3.3 TANGENTIAL ABRASIVE DEHULLING

Dehulling by TADD is achieved by a self-adhesive abrasive plate mounted on a 10" aluminum backing disk (Figure 4). Bottomless sample cups are used to contain the grain which sits on the rough abrasive surface. A minimum gap between cups and the abrasive surface can be adjusted by shims placed between the hub and the backing disk. During the operation, the hinged lid is closed to keep the seeds in the cups. A timer automatically controls the operating cycle during which the grains move around freely in the sample cups, and are peeled as they touch the abrasive surface. While the TADD is running, a gentle air flow caused by the high speed of spinning abrasive stone carries bran out the exhaust port connected to a bran collection device (Figure 5). The

three/quarter horsepower motor is equipped with a brake which stops the rotating disc instantly after the cycle time has ended. Dehulled grain samples can be removed from the sample cups into individual containers with a vacuum aspirating device supplied with the TADD (Figure 6).

The moisture content of Intrada wheat grain was adjusted to 15 % and 20 % in a tempering instrument (GE A-G Gear Motor, MOD: 14PCP4265, Ford Wayne, IN). A TADD (Venables Tangential Abrasive Dehulling Device, Model no. 4E-10/220, Veenables Machine Works, Ltd, Saskatoon, Canada) was used to remove outer layers from the whole wheat grain. The TADD could hold five bottomless cups; hence abrade up to five samples simultaneously. Each cup had the same dimensions, 6.23 cm inner diameter × 3.49 cm deep and could hold about 97 g seed. The abrasive plate (Coarse disc, Type 4, Perten Instruments, Huddinge, Sweden) was placed on a 10" aluminium support. The distance between the abrasive plate and the bottom edge of the sample cups was set at 0.042 inch. During the experiments each cup contained about 80 g of sample. Experiments were carried out at 1, 3 and 5 min abrasion time and three grain moisture levels, 15 %, 20 % and 11 % (the original wheat moisture level). Bran fractions obtained from TADD processing which were referred to as “TADD samples” throughout this dissertation were used for further testing.

3.4 WHOLE GRAIN SAMPLE PREPARATION

Approximately 100 g of whole grain samples were brought to room temperature prior to grinding. The grain samples were ground 3 times in 30 s intervals using a coffee grinder (Black & Decker CBG5, Miami, FL) at medium speed. The ground samples were

stored in plastic Ziploc bags at - 20 °C until further analyses.

3.5 EXTRACTION

Ten grams of TADD, bran, aleurone, and whole wheat samples were extracted separately with 100 mL of ethanol (80 %, v/v in water) for 16 h in dark under nitrogen. Extraction was repeated twice. The final concentration of the extract was determined by evaporating the solvent from 10 mL extract under vacuum and measuring the weight of residual extract.

3.6 MOISTURE CONTENT

The moisture content of the samples was determined by American Association of Cereal Chemists (AACC) method number 44-15A (AACC, 1995). Bran, TADD, whole grain, and aleurone 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 g of sample was weighed in a dried aluminum dish. Then the sample was dried in the oven at 130 °C for 1 h. The difference between the final and initial sample weight as percent of the initial sample weight was reported as the moisture content.

3.7 STARCH CONTENT

A starch determination kit (Megazyme International Ireland Ltd., Bray, Ireland) was used to analyze the starch content of the samples according to the Association of

Official Analytical Chemists (AOAC) method 996.11(AOAC 2005). One hundred mg of sample was weighed into a test tube. Aqueous ethanol (80 % v/v), 0.2 mL, was added in to the tube. Then the solvent and sample mixture was stirred on a vortex mixer. Dimethyl sulphoxide (DMSO), 2 mL, (ACS grade, Ameresco, Solon, Ohio) was immediately added to the same tube. Once again the mixture was stirred on a vortex mixer and then the tube was placed in boiling water for 5 min. After the addition of α-amylase solution (3000 U / mL) the solution was incubated in boiling water for 6 min. The tube was transferred into a water bath at 50 °C, after adding 0.1 mL of amyloglucosidase (3300 U / mL) and the tube content was incubated for 30 min. The final solution was diluted to 100 mL with DI water. An aliquot of this solution was centrifuged at 3000 rpm for 10 min. The upper layer of the centrifuged solution was used for the starch assay. An aliquot, 0.1 mL, of the solution was transferred in to a glass tube and 3.0 mL of glucose determination reagent including potassium phosphate buffer (0.26 M, pH 7.4), p-hydroxybenzoic acid (0.22 M) and sodium azide (0.4 % w/v), was added in to the same glass tube and the tube was incubated at 50 °C for 20 min. D-glucose was used as control and deionized water was used as blank. The absorbance of the samples was measured at 510 nm. Starch content was calculated as follows:

$$\text{Total starch (\%)} = \text{Abs} \times F \times FV / 0.1 \times 1 / 1000 \times 100 / W \times 162 / 180$$

$$= \text{Abs} \times FV \times F / W \times 0.9$$

Where;

Abs = absorbance of the solutions measured against reagent blank at 510 nm

F = factor to convert absorbance values to μ glucose = 100 μg glucose / absorbance value for 100 μg glucose;

FV = Final volume

3.8 ASH CONTENT

The ash content of the samples was determined according to the AOAC method 923.03 (AOAC, 1995). The 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 h at 525 °C and then cooled to room temperature in a desiccator. Approximately 2 g of samples was weighed into a dried crucible then sample was ashed in a furnace for 5 h at 525 °C. The percentage residual weight in the crucibles was reported as the ash content in the sample.

3.9 LIPID CONTENT

Lipid content was determined according to the AOAC method 960.39 (AOAC, 1995). Approximately 2 g of sample was weighed into a cellulose thimble. The thimble was 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 oil from the sample. The extraction time was 30 min. Extracted oil was dried in a vacuum oven (Fisher Scientific, Isotemp® Oven, Fair Lawn, NJ) for 15 min. The amount of extracted oil was determined gravimetrically.

3.10 PROTEIN CONTENT

Protein content of sample was analyzed as nitrogen on a LecoTruSpec carbon-nitrogen analyzer (TruSpec CN, Leco USA, St. Joseph, MI) according to the method of

National Forage Testing Association (NFTA, 2007). A factor of 6.25 was used to convert nitrogen to protein.

3.11 MINERAL CONTENT

The samples were ashed as described in section 3.8 of this dissertation. Minerals were extracted from ash in hot 3 N HCl before analysis using an Inductively Coupled Plasma (ICP) spectrometer (SpectroCiros, Fitchburgh, MA).

3.12 DPPH RADICAL SCAVENGING CAPACITY ESTIMATION

DPPH test was carried out on wheat extracts. Prior to absorbance measurements 2 mL of 60 µM DPPH solution (Sigma-Aldrich, St. Louis, MO, USA) was added to 50 µL of extract. Absorbance of the sample was recorded on a DU520 general purpose UV/Vis spectrophotometer (Beckman, Brea, CA, USA) at 517 nm for 60 min at 30 s intervals. The percent radical inhibition at 60 min is determined using the following equation:

$$\% \text{ DPPH inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where,

A_{sample} = absorbance of the sample at 60 min,

$A_{control}$ = the absorbance of control at time 0 min.

3.13 TOTAL PHENOLIC CONTENTS

A mixture of 0.1 mL of extract, 500 µL of FCR (Sigma-Aldrich, St. Louis, MO, USA), 1.5 mL of 20 % sodium carbonate, and 7.9 mL deionized water was allowed to react for 2 h at ambient temperature. Absorbance of the solution was measured at 765 nm

on a DU520 UV/Vis spectrophotometer (Beckman, Brea, CA, USA) at time 2 h. Gallic acid (Sigma - Aldrich, St. Louis, MO, USA) was used to prepare an external standard curve for quantification. Total phenolic content of the samples was reported as gallic acid equivalents (GE) per g of solid sample used for extraction.

3.14 OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) ASSAY

The ORAC assay was performed by using a HTS-7000 Microplate (Perkin-Elmer Co., Covina, CA, USA) reader at excitation and emission wavelengths of 485 and 535 nm, respectively. All the reagents were prepared in pH = 7.0 phosphate buffer. Extract, 20 µL, was mixed with 160 µL of fluorescein (TCI, Portland, OR, USA) (0.2 µg / mL) and 20 µL AAPH (Sigma-Aldrich, St. Louis, MO, USA) (33.2 mg / mL) in a 48-well clear plate and reacted at 37 ºC for 45 min. The same procedures were used to prepare blank and standard which were 20 µL of PB (pH = 7.0) and 20 µL of 10 µM Trolox (Sigma-Aldrich, St. Louis, MO), respectively. ORAC values were calculated as:

[(AUC sample - AUC blank) / (AUC Trolox - AUC blank)] × dilution factor (DF) × (µmoles Trolox / g sample).

ORAC value was expressed as µmoles Trolox / g sample.

3.15 TOCOPHEROLS AND TOCOTRIENOLS ANALYSES

Tocopherol and tocotrienol contents of the samples were analyzed according to Katsanidis and Addis (Katsanidis and Addis 1999). The HPLC system (Alliance 2690 Waters Corp., Milford, MA, USA) consisted of a separations module (Model 2695) and a Photodiode Array Detector (PDA) (Model 2996, Waters, Milford, MA, USA). Extracts

were filtrated through a 0.45 μm syringe filter before injecting to the HPLC. Two microliter of sample was injected into a normal phase HPLC column, Zorbax RX-SIL (5 μm particle size, 4.6 × 250 mm, Agilent Technologies, Santa Clara, CA, USA) and separation was achieved by using a mobile phase consisting of hexane (HPLC Grade, Fisher Scientist, Fairlawn, New Jersey, USA) and isopropanol (HPLC grade, Pharmco Co. Brookfield, CT, USA) at a ratio of 99:1. Isocratic flow rate was 1.3 mL / min. Column temperature was set at 35 °C. The tocopehrols and tocotrienols compositions of the samples were identified by direct comparison of their chromatographic retention times with those of the authentic compounds. An external calibration curve was used to quantify tocol content of the extracts. Seven individual tocol standards, α-tocopherol (α-T), β-tocopherol (β-T), δ-tocopherol (δ-T), γ-tocopherol (γ-T), α-tocotrienol (α-T3), δ-tocotrienol (δ-T3) and γ-tocotrienol (γ-T3) were purchased from Sigma (Sigma-Aldrich Corporation, St. Louis, MO) and used for peak identification. All the samples were prepared and analyzed in triplicate. The data collection and analysis were managed using Waters Pro Empower software (Version 5.00.00.00, Waters Corp., Milford, MA) running on a PC (DELL, XP-Professional, Round Rock, TX).

3.16 PHENOLIC ACIDS ANALYSES

Phenolic acids in samples were analyzed by a Waters 2695 series HPLC (Alliance 2690 Waters Corp., Milford, MA, USA) equipped with a Photodiode Array Detector (PDA) (Model 2996, Waters, Milford, MA, USA) and a SunFire C18 (250 × 4.60 mm, 5 μm, Phenomenex, Torrance, CA) column. After filtered through a 0.45 μm syringe filter, 10 μL of sample was injected to the HPLC. A mobile phase consisting of

acetonitrile (solvent A) (HPLC grade, VWR, West Chester, PA, USA) and 2 % acetic acid in water (v / v) (solvent B) was used at a flow rate of 1.0 mL / min for a total run time of 70 min. The solvent gradient program was as follows: 100 % B to 85 % B in 30 min, 85 % B to 50 % B in 20 min, 50 % B to 0 % B in 5 min and 0 % B to 100 % B in 15 min. Benzoic and cinnamic acids were monitored at wavelengths, 280 and 320 nm, respectively. Individual phenolic acids were identified by comparing the retention time of the sample peaks with that of the standards. Gallic, benzoic, vanillic, caffeic, syringic, *p*-coumaric and ferulic acids from Sigma (Sigma-Aldrich Corporation, St. Louis, MO, USA) were used as standards. External calibration curves prepared for individual acids were used to quantify the amount of phenolic acids in the samples. All samples were prepared and analyzed in triplicate. Data signals were acquired and processed on a PC (DELL, XP-Professional, Round Rock, TX) running the Waters Pro Empower software (Version 5.00.00.00, Waters Corp., Milford, MA).

3.17 CAROTENOIDS ANALYSES

The carotenoid analysis was conducted by using a Waters HPLC system equipped with a Waters 2487 dual-wavelength absorbance detector, a Waters 600S controller, a Waters 616 pump, an inline degasser, and a Waters 717 autosampler (Waters Corp., Milford, MA). Mobile phase consisted of HPLC grade methanol (Fisher Scientist, Pittsburgh, PA, USA) and deionized water from a Millipore water purification system (Millipore Corporation, Molsheim, France)at a ratio of 95:5 as solvent A and HPLC grade Methyl tert-butyl ether (MTBE) (Mallinckrodt Baker, Inc. Phillipsburg, NJ, USA) as solvent B. Isocratic elution was achieved with 75 % solvent A and 25 % solvent B.

Sample, 10 µL, was injected to YMC Carotenoid S-3 column (250 × 4.6 mm, 3 µm column, Waters Corp.) at an isocratic flow rate of 1.9 mL / min. Column temperature was set at 35 °C. The individual carotenoids standards, lutein, zeaxanthin and β-carotene from Sigma (Sigma-Aldrich Corporation, St. Louis, MO, USA) were detected at 450 nm. External calibration curves were used to quantify carotenoids in the samples. The individual carotenoids were identified by direct comparison of the retention times of the peaks appeared in sample chromatogram with those of the authentic compounds. All samples were prepared and analyzed in triplicate. Data signals were acquired and processed on a PC (DELL, XP - Professional, Round Rock, TX) running the Waters Empower2 software (Version 6.20.00.00, Waters Corp., Milford, MA).

3.18 ORGANIC ACIDS ANALYSES

The organic acids analysis was performed by using an Agilent 1200 Series HPLC system. The mobile phase, 0.005 M sulfuric acid, was degassed by a Degasser (G1322A, Agilent, Santa Clara, CA, USA) by using a Quad Pump (G1311A, Agilent, Santa Clara, CA, USA). A Bio-radAminex HPX - 87H column (30 × 7.8 mm, Bio-rad, Hercules, CA, USA) equipped with a guard column (30 × 4.6 mm, Bio-rad, Hercules, CA, USA) were used for the analysis. Sample, 5 µL, was injected by an autosampler (ASL, G1329A, Agilent, Santa Clara, CA, USA). Flow rate was 0.6 mL/ min. Column temperature was 35 °C. Dual-wavelength absorbance detector (G1315D, Agilent, Santa Clara, CA, USA) was used to measure the absorbance of organic acids at 210 nm. The organic acid peaks appeared on the sample chromatograms were identified by direct comparison of their chromatographic retention times with those of authentic compounds. An external

calibration curve was used to quantify each organic acid in samples. Citric, ascorbic, malic, succinic, lactic and fumaric acids (Sigma - Aldrich, St. Louis, MO) were used as standards. All samples were prepared and analyzed in triplicate. Data signals were acquired and processed on a PC (DELL, XP-Professional, Round Rock, TX) running the ChemStation for LC 3D software (Rev. B.04.0x. Agilent Technologies, and Palo Alto, CA).

3.19 POLICOSANOL AND PHYTOSTEROL ANALYSES

3.19.1 Hydrolysis

Approximately 2 g of ground sample was hydrolyzed by refluxing with 30 mL 1.0 N NaOH in methanol for 45 min while stirring. Then solution was cooled and filtered through glass wool using a glass funnel. Residual material on the glass wool was washed with deionized water from a Millipore water purification system (Millipore Corporation, Molsheim, France) 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 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 1 mL volumetric tube. Then 250 µL silylation reagent [N-Methyl-N- (trimethylsilyl) trifluoroacetamide] (MSTFA), Pierce (Rockford, IL)] and 500 µL chloroform was added to the tube. The sample was derivatized by heating at 60 °C for 15 min. The total volume was brought to 1 mL by chloroform before analysis using a GC.

3.19.2 GC Analysis

PC and PS contents of the samples were analyzed by using a HP 6890 Series GC system coupled with a flame ionization detector (FID) (HP Company, Wilmington, DE). A fused silica capillary Equity-5 column (30 m × 0.25 mm × 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 °C for 15 min. 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 peaks appeared on the sample chromatograms 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 % purity) 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 an 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 min. The desired concentrations of standard solutions were prepared by dilution of the stock solutions.

3.20 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

For TADD experiments a 3 × 3 factorial (moisture by time) design was used. Three references (aleurone, Intrada bran, and whole wheat) were also analyzed. The antioxidant capacity of TADD samples were compared to the aleurone sample by using Dunnett's multiple comparison method. Samples having significantly higher or similar antioxidant capacity as the aleurone were further analyzed for their bioactive compounds. All the tests were carried out at least in duplicate and in randomized order with the mean values being reported. The means were compared using Tuckey's adjustment. Experimental data were assessed for non-normality and heterogeneity of variances. Appropriate linear and generalized linear mixed models were analyzed using version 9 SAS/GLM, SAS/MIXED and SAS/GLIMMIX procedures (Software Version 9.2. SAS Institute INC., Cary, NC). All statistical tests were performed at the 0.05 level of significance.

CHAPTER 4

RESULTS

In this study two sets of samples were examined. The first set of samples consisted of bran fractions obtained by using a TADD at various grain moisture contents and abrasion times. These samples were labeled as follows: TADD - Moisture content of the sample - Abrasion time. For example "TADD-20-3" refers to the TADD sample obtained at 20 % grain moisture content and 3 min TADD abrasion time. All TADD samples were from wheat variety Intrada grown in Oklahoma. In previous studies it was found that Intrada had significantly higher amount of bioactive compounds than the other varieties examined (Chen and others 2009a; Chen and others 2009b). The second set included a commercial aleurone sample and a "bran" sample obtained by using Intrada wheat variety and a quadrumat milling system as explained in the experimental section of this dissertation. Information on wheat varieties used to produce commercial aluerone was not available. Whole wheat sample was the same variety as TADD samples, Intrada.

4.1 TADD YIELD

In this dissertation the term "TADD yield" refers to the bran collected by using a TADD. TADD yield varied from 6.76 % to 31.03 % (w/w, dry basis) depending on the moisture content of the sample and the abrasion time (Table 1). Effects of moisture × time on TADD yield was significant ($p < 0.0001$). At all sample moisture levels TADD yield increased as the abrasion time increased. Both the lowest and the highest TADD

yields were obtained at 20 % sample moisture and 1 and 5 min abrasion times, respectively (Table 1, samples TADD-20-1 and TADD-20-5). It appears that grain tempering with excess water caused over saturation of the outer grain layers. As a result bran removal was not efficient at very short abrasion time.

4.2 STARCH CONTENT

Starch content of the samples ranged from 2.47 % to 50.16 % (w/w, dry basis), with aleurone and whole wheat having the lowest and the highest starch contents, respectively (Table 2). High starch content in whole wheat was expected. Because starch is the main component of endosperm and whole wheat samples did not go through a bran separation process. Effect of moisture by abrasion time interaction on starch content was significant ($p = 0.0002$). For TADD samples, the starch content increased significantly as the abrasion time increased, except for TADD-20-3 and TADD-20-5. Among all the samples examined in this study commercial aleurone sample had the lowest starch content (2.47 %) indicating very effective endosperm separation during production. As expected whole wheat grain had very high starch content (50.16 %). Bran also had significant amount of starch, 18.86 %.

4.3 PROTEIN CONTENT

The highest protein content among the TADD samples was obtained at 15 % moisture and 1 min abrasion time (Table 3). TADD-11-5 had the lowest protein content (13.61 %). Effect of moisture × time ($p < 0.0001$) on protein content of the samples was significant. Protein content of this sample was similar to that of the bran ($p = 0.2764$).

Our test results on protein content of bran (20.52 %) were in agreement with the literature values (14.2 - 26.2 %) (Hidalgo and Brandolini 2008). Protein content of the whole wheat was within the range reported in the literature 10 - 18 % (Šramková and others 2009) and similar to that reported for Intrada wheat (Chen and others 2009a).

4.4 LIPID CONTENT

Lipids are present in small amounts in whole wheat and flour. They play an important role in processing, storability, nutrition, and consumer acceptance of the flour based products (Konopka and others 2004).

Table 4 shows the lipid content of the samples as affected by moisture and abrasion time. Lipid content ranged from 2.92 % to 4.57 % (w/w, dry basis). Effect of moisture × abrasion time on lipid content was significant ($p < 0.0001$).Whole wheat had significantly lower lipid content than the other samples. This result is similar to the data reported by Konopka and others (2004). The reason for low lipid content was because of the presence of large amount of endosperm which diluted lipids in whole wheat. The lipid contents of bran and aleurone determined in this study were slightly lower than those reported by Khan and Shewry (2009).

4.5 ASH CONTENT AND MINERAL COMPOSITION

Ash content is associated with the mineral content of wheat grain and varies with genotype and growth environment (Chen and others 2009a). Minerals are concentrated in the outer layers of the grain. High ash content indicates the presence of bran in the

samples. Endosperm or flour fraction of wheat is not a good source of minerals (Varo and others 1980).

The ash contents of the samples are shown in Table 5. Aleurone had the highest ash content, 7.40 % (w / w, dry basis), among the samples examined in this study. This finding supports the starch content data discussed earlier in section 4.2 of this dissertation. Commercial aleurone sample had the lowest starch content indicating good starch separation during fractionation. Effect of moisture × abrasion time on ash content was significant ($p < 0.0001$). Whole wheat had significantly lower ash content (1.66 %, w/w, dry basis) due to the presence of abundant flour. Similar to the findings of this dissertation Hidalgo and Brandolini (2008) reported that bran had much higher ash content than whole wheat. Nine minerals, Na, Mg, P, K, Ca, Fe, Cu, Zn and Mn, were detected in all the samples. Mg, P, K and Ca were the major minerals in all the samples (Table 6). Findings of this study were consistent with other studies (Khan and Shewry 2009).

4.6 EXTRACTION YIELD

All wheat samples were extracted with ethanol (80 %) / water (20 %) mixture. Yield and concentration of the extracts are shown in Table 7. Extraction yield which was expressed as weight percentage of the sample ranged from 12.83% to 6.97%. Effect of moisture × abrasion time on extract yields was significant ($p < 0.0001$). TADD-20-1 and whole wheat extract yields were similar ($p = 0.9810$) but significantly lower than those for the rest of the samples. Low extraction yield for whole wheat was expected because of the large endosperm content in the sample. It is conceivable that all TADD samples

contained some germ except TADD-20-1 because of the very low TADD yield obtained with the latter sample (Table 1).

4.7 ANTIOXIDANT CAPACITY ANALYSES

In this study, three antioxidant capacity tests, ORAC, TPC and DPPH were used to evaluate the wheat extracts. Antioxidant capacity of all the TADD extractss was compared to that of the commercial aleurone extract by using Dunnett test. Those samples which had similar to or significantly higher antioxidant capacity than aleurone were further analyzed for their content and composition of bioactive compounds. Butylated hydroxytoluene (BHT), a synthetic antioxidant commonly used in food applications, was used as a reference.

4.7.1 OXYGEN RADICAL ABSORBANCE CAPACITY

All the wheat extracts examined in this study exhibited strong peroxyl radical scavenging ability. TADD-11-1, TADD-20-3 and aleurone had the highest ORAC values among the wheat samples (Table 8). Extracts from all nine TADD samples had higher ORAC values than the bran and whole grain extracts. Effect of moisture × time interaction on ORAC values was significant ($p < 0.0001$). Bran and whole wheat had similar ORAC values. Iqbal and others (2007), and Moore and others (2005) reported higher ORAC values for bran and whole grain than those reported in this dissertation. Use of different extraction solvents and protocols may cause this variation. Iqbal and others (2007) used methanol extraction first then extracted the residue with 2 moles/L sodium hydroxide again, and Moore and others (2005) extracted antioxidants by using a

50 - 50 acetone-water mixture. Zhou and others (2004a) have demonstrated that acetone-water extraction resulted in greater ORAC value than the ethanol extraction. However, ORAC value for bran reported in this study was similar to that reported by Yu and others (2005) by using absolute ethanol. BHT had significantly higher ORAC value than all the samples.

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4.7.2 TOTAL PHENOLIC CONTENT

Table 8 shows the TPC results for the samples examined in this study. Whole wheat, TADD-15-5 and TADD-20-1 had the lowest TPC (1.43-1.86 GE mg / g sample) which was almost half of the TPC for bran (2.41 GE mg / g sample). Liyana-Pathirana and Shahidi (2007a) also reported that bran had 2-3 times more TPC than whole grain. Moisture × time interaction had a significant effect on TPC ($p < 0.0001$). TADD samples obtained at 1 min had high TPC values that were similar to that of bran and aluerone except TADD-20-1. This result was due to low bran recovery at 20 % grain moisture level caused by oversaturation of the samples and stickiness of endosperm and bran. BHT had significantly higher TPC value than all the other samples.

Adom and Liu (2002) evaluated total phenolic content of four cereal grains, corn, wheat, oats and rice. Corn had the highest TPC followed by wheat (1.36 mg GE / g of grain) which was similar to the value reported in this study. Similar results have also been reported by other research groups (Iqbal and others 2007; Zhou and Yu 2004a).

4.7.3 DPPH

Table 8 shows DPPH radical scavenging ability of wheat extracts. Whole wheat exhibited the lowest DPPH inhibition (9.79 %). BHT had significantly stronger DPPH radical scavenging ability than all the samples. Moisture × time had a significant effect on DPPH ($p < 0.0001$).

It has been reported that wheat showed lower DPPH quenching ability than that of black rice, sorghum and barley (Choi and others 2007). In this dissertation, bran had significantly stronger DPPH scavenging ability than whole grain ($p < 0.0001$) which was also reported by Liyana-Pathirana and Shahidi (2007a). Zhou and Yu (2004b) examined DPPH inhibition ability of Trego and Akron wheat varieties. Ethanol extracts (70% ethanol) of Trego and Akron whole grain scavenged 46.56% and 37.83% of DPPH radicals, respectively. In a following study, bran extracts from Trego and Akron wheat collected from three locations in Colorado were examined and found that 52.47- 60.03 % and 72.14 - 72.48 % of DPPH radicals were quenched by Trego and Akron bran extracts, respectively (Yu and others 2005).

A statistical analysis of the results obtained from three antioxidant capacity tests examined in this study, TPC, DPPH and ORAC, indicated that TADD-11-1, TADD-15-1, TADD-15-3, TADD-20-3, TADD-20-5 had higher antioxidant capacity than the other TADD samples. Therefore, these samples were further analyzed for their chemical composition focusing on the health beneficial bioactive compounds. Aleurone, bran and whole wheat samples were also analyzed for their chemical composition as references.

4.8 TOCOPHEROLS AND TOCOTRIENOLS

Table 9 shows the tocols content and compositions of the samples. Whole wheat had the lowest tocols content, 3.02 µg / g sample. Aleurone (12.20 µg / g sample), TADD-20-3 (11.02 µg / g sample), and TADD-15-1 (10.47 µg / g sample) showed significantly higher tocols than the other samples. All TADD sample had higher tocols content than the bran sample. The tocols content of bran sample was in accordance with other studies (Zhou and others 2005; Zhou and others 2004b). These results support earlier reports demonstrating that tocols are concentrated in the outer layers of wheat grain (Hidalgo and Brandolini 2008).

HPLC method used in this study did not separate α-tocopherol from α-tocotrienol; hence the result was expressed as α-tocopherol + α-tocotrienol. Similarly γ-tocopherol and γ-tocotrienol peaks overlapped on the sample chromatograms; therefore, results for these compounds were reported as γ-tocopherol + γ-tocotrienol. The tocol compositions varied among the samples. α-Tocopherol + α-tocotrienol and γ-tocopherol + γ-tocotrienol were detected in all TADD and reference samples except whole wheat (Table 9). Only α-tocopherol + α-tocotrienol peak was identified in the whole wheat. β- and δ-Tocopherols were found only in the aleurone sample. In a study of Maryland-grown soft wheat bran, only α-tocopherol, ranging from 3.4 µg / g to 10.1 µg / g, was reported (Moore and others 2005). Zhou and others (2005) examined the tocopherol content and compositions in hard red winter wheat bran and reported the presence of α-tocopherol (4.10 - 6.51 µg / g), δ-tocopherol (0.16 - 0.38 µg / g) and γ-tocopherol (3.68 - 5.59 µg / g). These results are in agreement with the data presented in this dissertation. TADD-15-1, TADD-20-3 had significantly higher α-tocopherol + α-tocotrienol as well as γ-tocopherol

+ γ-tocotrienol content than the other samples. Aleurone also contained significant amount of α-tocopherol + α-tocotrienol. Tocols are lipid soluble antioxidants that are concentrated in bran and germ. TADD-20-3 and aleurone contained significant amount of lipid. Extraction yield for TADD-15-1 was higher than the other samples leading to higher antioxidant concentration in the sample. These findings are in agreement with the high tocol content in TADD-20-3, TADD-15-1 and aleurone samples.

4.9 CAROTENOIDS

In this study, all the samples were analyzed for their carotenoid compositions including β-carotene, lutein and zeaxanthin. The total carotenoid content of the samples ranged from 1.89 to 3.83 μg / g sample (Table 10). Whole wheat had significantly lower carotenoid content than the other samples due to the large quantity of endosperm. TADD-15-1, TADD-15-3, TADD-20-3, and TADD-20-5 had significantly higher total carotenoids as well as lutein contents than the aleurone. The major carotenoid in cereal grains is lutein, followed by zeaxanthin (Konopka and others 2006). In this dissertation, only lutein and zeaxanthin were detected in all the samples (Table 10). Lutein was the primary carotenoid in all the samples ranging from1.41 to 2.92 μg / g sample. This range is comparable to the values reported in earlier studies (Hidalgo and others 2006; Zhou and others 2004b). The amount of lutein in the samples is almost two to three folds that of zeaxanthin (0.48-0.95 μg / g sample). Similar results were also reported by other research groups (Zhou and others 2004b; Zhou and others 2005; Moore and others 2005). Similar to the findings of this study, Adom and others (2005) reported that wheat bran had significantly higher carotenoids content than endosperm.

4.10 PHENOLIC ACIDS

In cereal grains phenolic acids are present in three forms: free, soluble-conjugated and bound. Most phenolic acids exist in a bound form. Only free phenolic acids can be extracted by aqueous ethanol (Adom and Liu 2002).

Table 11 shows the free phenolic acid contents and compositions in the samples. Whole wheat had significantly lower total phenolic acids (16.33 µg / g sample) than other samples mainly due to the presence of endosperm. TADD-20-3, TADD-11-1, TADD-15-3 and aleurone had the highest total phenolic acids content among the samples (41.21-47.23 µg / g sample). TADD-11-1, TADD-15-3, and TADD-20-3 had significantly higher phenolic acids contents than bran (39.51 µg / g sample), but not significant from the commercial aleurone sample (45.11 µg / g sample). It has been reported that free phenolic acids were present in the wheat germ, the main reservoir of lipids (King, 1962). TADD-11-1, TADD-15-3, and TADD-20-3 contained significantly higher amount of lipid than bran. Hence three TADD fractions with high oil content also had higher phenolic acids than bran.

Benzoic, vanillic, caffeic, syringic, *p*-coumaric, and ferulic acids were found in all the samples (Table 11). Ferulic, benzoic, syringic, and vanillic were the major phenolic acids extracted from wheat samples. TADD-11-1 and aleurone contained the highest ferulic acid contents, 14.98 µg / g sample and 12.87 µg / g sample, respectively. There was no significant difference between TADD-11-1 and aleurone ($p = 0.3007$), but TADD-11-1 showed significantly higher ferulic acid content than the rest of the samples.

Free phenolic acids are a small portion of the total phenolic acids in wheat (Sosulski and others 1982). Kim and others (2006) examined four types of wheat bran

and found that free phenolic acid content in the samples ranged from 3.87 to 31.13 µg / g bran. It was also reported that ferulic, vanillic and syringic acids were the major free phenolic acids in bran. These results were comparable to our study. Adom and Liu (2002) reported that free ferulic acid in the wheat sample was 1.11 µg / g grain which was lower than that found in our samples. It is important to note that Adom and Liu used dehulled.

4.11 ORGANIC ACIDS

Total organic acid contents of the samples are shown in Table 12. Whole grain had the lowest organic acid content (29.79 µmole / g sample). TADD-15-1 (82.50 µmole / g sample) and bran (76.64 µmole / g sample) had significantly higher amount of organic acids than other samples. All the TADD samples had significantly higher organic acids than the aleurone sample indicating that organic acids are concentrated in the bran fractions. HPLC method used to analyze organic acids could not separate ascorbic and malic acids. Hence results were reported as ascorbic + malic acids. Citric, ascorbic + malic, succinic, and fumaric acids were found in all the samples (Table 12). Succinic, citric, and fumaric acids were the most abundant organic acids in the samples. In aleurone, the succinic acid comprised approximately 73 % of the total organic acids. Literature on organic acids contents and compositions of wheat components are scarce. Organic acid content and composition in plants vary with plant type and genotype (Clark 1969). Most of the studies on organic acids in wheat were carried out on wheat leaves (Burke and others 1985; Clark 1969). Lohaus and others (1983) pointed out that wheat kernel had much greater organic acids contents than those observed in wheat leaves. Malic, citric, succinic and fumaric acids were the major acids found in wheat, rye and barley. The

latter study reported higher amounts of organic acids in wheat as compared to this dissertation possibly due to the different extraction method used.

4.12 POLICOSANOL

The total PC content of the samples ranged from 21.70 to 64.81 µg / g sample (Table 13). Aleurone had significantly higher PC content than the other samples. TADD-11-1 and TADD-15-1 had significantly higher PC than other TADD samples. Nine PC components were identified in all the samples. C23, C24, C26, C28, and C30 were the most abundant PC components found in all the samples (Table 13).

A previous study on whole wheat showed that total PC content in Intrada variety varied from 16.0 to 26.2 µg / g sample and C23, C24, C26, C28, and C30 were the major PC components (Chen and others 2009b). The total PC content of whole wheat found in this study was consistent with the previous study. Irmak and Dunford (2005) examined PC content and compositions in the bran of 31 wheat varieties in Oklahoma. Intrada had significantly higher PC content, 37.0 µg / g, than the other wheat varieties examined. PC content of the bran examined in this study was higher than that reported in the latter study.

4.13 PHYTOSTEROL

Wheat contains significant amount of PS. Table 14 shows the total PS content of the samples. Whole wheat had significantly lower PS content, 320.93 µg / g sample, than the other samples. TADD-11-1 (1660.18 µg / g sample) had the highest PS content among the samples examined in this study. β-Sitosterol, stigmasterol, and campesterol were the major PS found in all the samples (Table 14), as β-sitosterol was the most

abundant PS representing over 60% of the total PS. TADD-11-1 had significantly higher β-sitosterol contents than rest of the samples. Aleurone had significantly higher stigmasterol and campesterol than the other samples.

The PS content and compositions of Intrada wheat has been evaluated by Chen and others (2009a). The PS content of the whole grain sample used in this study was consistent with the previous study (Chen and others 2009). It has been reported that wheat bran had higher PS content than whole grain (Nystrom and others 2007; Jiang and Wang 2005). A similar trend was observed in this dissertation. The PS content of the bran used in this dissertation is slightly lower than the previously reported values in the literature (Nystrom and others 2007; Piironen and others 2002). The latter studies used both acid and alkaline hydrolysis for PS analysis of the samples. In this dissertation, only alkaline hydrolysis was carried out.

CHAPTER 5

DISCUSSION

Starch content of the samples was positively correlated with TADD yield ($r = 0.90763$, $p < 0.0001$, where r refers to Pearson Correlation Coefficient) because of the increased weight of the sample collected from TADD as starch content increased. A negative correlation between ash content and TADD yield ($r = -0.91622$, $p < 0.0001$) suggests that bran concentration in the TADD samples decreased as TADD yield increased because of the increased starch amount which diluted bran fraction with high ash content.

Ash content in grain samples is a sign of the presence of bran. In this study, ash content was significantly correlated with tocols ($r = 0.77320$, $p < 0.0001$), carotenoids ($r = 0.41665$, $p = 0.0428$), and phenolic acids ($r = 0.62816$, $p = 0.0010$), indicating that these bioactive compounds are concentrated in bran fraction of the wheat. Since tocols and carotenoids are lipid soluble antioxidants, they are significantly correlated with the lipid content ($r = 0.85981$, $p < 0.0001$; $r = 0.82959$, $p < 0.0001$). Additionally, phenolic acids were also positively correlated with the lipid content of the samples ($r = 0.89669$, $p < 0.0001$). King (1962) reported that free phenolic acids were found in the wheat germ. It is expected that samples with high oil content will comprise greater amount of germ that also contains phenolic acids.

Three different antioxidant capacity tests were carried out in this study：ORAC, TPC, and DPPH. The goal was to examine the different reaction mechanisms that might be involved in the action of wheat extracts as antioxidants. ORAC assesses the hydrogen atom donation ability of the extracts to quench peroxyl radicals, TPC expresses the total phenolic content, which contributes to the antioxidant capacity of the samples, and DPPH measures the electron transfer ability of the extracts to inhibit organic nitrogen radicals. ORAC values were positively correlated with the lipid ($r = 0.65181$, $p < 0.0001$) and ash contents of the samples ($r = 0.60509$, $p < 0.0001$). The strong correlation between ORAC and the lipid content is due to the presence of lipid soluble antioxidants such as tocols and carotenoids in the extracts. These compounds are effective peroxyl free radical scavengers. Strong correlation between ORAC and tocols content of the samples ($r = 0.75793$, $p < 0.0001$) also supports the peroxyl radical scavenger properties of the extracts. Phenolic acids were significantly correlated with ORAC ($r = 0.65813$, $p < 0.0001$) as well. Phenolic acids are effective peroxyl radical scavengers because of their hydrogen atom donation ability (Yeh and Yen 2003). Both ferulic acid/ORAC and α-tocopherol + α-tocotrienol/ORAC correlations were strong and positive. Among the bioactive compounds, ferulic acid was the highest contributor to the ORAC values (Table 15) ($r^2 = 0.6282$, where r^2 refers to square of Pearson Correlation Coefficient). Ferulic acid is a potent antioxidant due to its phenolic nucleus and unsaturated side chain (Castelluccio and others 1996). The –COOH substitution on the phenol ring makes it easier for the ferulic acid to donate its hydrogen atom (Nenadis and others 2003).

Ash content was positively correlated with DPPH ($r = 0.53862$, $p = 0.0007$) due to the same reason as discussed earlier. Correlations between ORAC-DPPH, ORAC-TPC,

TPC-DPPH were all significant ($p < 0.0001$) and positive. The strongest correlation was observed between TPC and DPPH, $r = 0.71478$. Similar findings have been reported by other research groups (Zhou and others 2004a; Zhou and others 2004b). DPPH has been widely used to evaluate the radical scavenging ability of phenolic compounds which are strong quenchers of the DPPH radicals (Villaño and others 2007; Fauconneau and others 1997). Phenolic acids have both hydrogen donating and electron transfer ability (Rice-Evans and others 1996; Rice-Evans and others 1997). DPPH radicals react with phenolics via two different mechanisms. In these reactions solvent type plays a key role. The HAT mechanism is dominant if the reaction takes place in apolar solvent while polar solvents such as methanol or ethanol leads to ET mechanism (Foti and others 2004). Tocols, carotenoids, and phenolic acids were shown to have a significant positive correlation with DPPH assay (Prior and others 2005). Tocols and carotenoids can stop oxidation by donating a hydrogen atom to a free radical (Niki and others 1984; Gurney and others 1996; Terao 1989). Ferulic acid showed strong DPPH radical inhibition properties ($r^2 = 0.5497$) (Table 16). As expected phenolic acid content was positively correlated with TPC ($r = 0.83516$, $r < 0.0001$). Caffeic acid was the most important compound affecting the TPC result ($r^2 = 0.8750$) (Table 17).

Although PC and PS do not contribute to the antioxidant capacity of wheat extracts they were examined in this study because of their putative health benefits. PC content was significantly correlated with ash content ($r = 0.81425$, $p < 0.0001$) because PC was concentrated in the bran fraction of wheat grain (Irmak and Dunford 2005). PC is a group of compounds that are classified as wax and PS are normally found in the oils. This could be the reason that both PC and PS were significantly correlated with the lipid

content ($r = 0.80203$, $p = 0.0002$; $r = 0.63741$, $p = 0.0079$).

CHAPTER 6

CONCLUSION

This study examined efficiency of the tangential abrasive dehulling method to produce wheat fractions enriched in health beneficial bioactive compounds. TADD-20-3 contained significant amounts of tocols, carotenoids and phenolic acids. TADD-15-1 contained the most organic acids. TADD-11-1 had the highest PC and PS content. Most TADD fractions expressed greater antioxidant capacity and contained higher amount of bioactive compounds than bran. Whole wheat had fewest bioactive compounds content due to the existence of large amount of endosperm. The results indicated that TADD is an efficient processing method to enrich bioactive compounds in wheat extracts. Both moisture and abrasive time affected the antioxidant capacity of the samples. TADD-11-1, TADD-15-1, TADD-15-3, TADD-20-3, and TADD-20-5 had the highest antioxidant capacity among the samples. This trend indicated that at low moisture levels, 11 % and 15 %, shorter abrasive time was required to produce fractions with higher antioxidant activity. However, at high moisture levels, 20 %, longer abrasive time was needed to recover bran because of the oversaturation and stickiness of wheat bran and endosperm fractions. It appears that short abrasion time is effective in minimizing starch content in TADD samples which dilutes the bioactive compounds in the final product. High moisture tempering (>15%) should be avoided prior to bran separation since it leads to

endosperm bran stickiness leading to poor separation efficiencies. This study clearly demonstrated that TADD produced products that have similar or higher antioxidant capacity as the commercial aleurone. Considering that the commercial sample had significantly lower starch content than that of the TADD samples, downstream processing of TADD products to remove starch (i.e. sieving process) could produce superior products to the commercial sample.

Bioactive compounds greatly contributed to the antioxidant capacity tests. Tocols and phenolic acids were strongly correlated with the ORAC values. DPPH was positively correlated with tocols, carotenoids and phenolic acids. Ferulic acid was the dominant compound contributing DPPH radical scavenging capacity of the extracts.

FUTURE WORK

Surface Response Methodology should be used to optimize TADD processing parameters to obtain wheat fraction with high antioxidant capacity. A sieving system should be added after TADD processing to minimize endosperm contamination in the samples and consequently increase the antioxidant capacity of the final product. Effect of solvent type on recovery of bioactive compounds from wheat fractions requires further research. An economic feasibility study is the key to determine market potential of wheat extracts as functional food ingredients, nutracueticals and dietary supplements.

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Table 1: TADD * yield [% (w / w), dry basis]

Sample	TADD yield
TADD - 11 - 1	10.50 ± 0.18^{d}
TADD - 11 - 3	18.66 ± 0.13^{c}
TADD - 11 - 5	28.81 ± 0.18^{a}
TADD - 15 - 1	11.13 ± 0.30^{d}
TADD - 15 - 3	23.09 ± 0.48^{b}
TADD - 15 - 5	28.28 ± 0.54^{a}
TADD - 20 - 1	6.76 ± 0.03^{e}
TADD - 20 - 3	18.93 ± 0.28^{c}
TADD - 20 - 5	31.03 ± 0.61^{a}
Bran	29.66 ± 2.81

*The sample abbreviations are as following:

For TADD samples: TADD - moisture content - abrasion time, i.e. TADD - 11- 1 refers to bran obtained by using TADD at 11% (original) moisture level, 1 minute abrasion time. Bran refers to the sample obtained by using a quadrumat senior mill.

[abcd] Sample means within a column that have the same letter are not significantly different

($\alpha = 0.05$)

Table 2: Starch content of wheat samples* [% (w / w), dry basis]

Sample	Starch content
TADD - 11 - 1	18.29 ± 0.53^{de}
TADD - 11 - 3	36.60 ± 0.31^{c}
TADD - 11 - 5	42.10 ± 1.00^{b}
TADD - 15 - 1	20.94 ± 0.50^{d}
TADD - 15 - 3	36.53 ± 0.33^{c}
TADD - 15 - 5	43.28 ± 1.09^{b}
TADD - 20 - 1	15.86 ± 0.41^{e}
TADD - 20 - 3	38.48 ± 0.70^{bc}
TADD - 20 - 5	37.75 ± 1.00^{bc}
Bran	18.86 ± 0.29^{de}
Whole wheat	50.16 ± 1.16^{a}
Aleurone	2.47 ± 0.06^{f}

*The sample abbreviations are as following:

For TADD samples: TADD - moisture content - abrasion time, i.e. TADD - 11- 1 refers to bran obtained by using TADD at 11 % (original) moisture level, 1 minute abrasion time. Whole wheat refers to the whole Intrada wheat sample. Bran and aleurone refer to the commercial samples.

[abcd] Sample means within a column that have the same letter are not significantly different (α=0.05)

Table 3: Protein content of wheat samples* [% (w / w), dry basis]

Sample	Protein content
TADD - 11 - 1	14.17 ± 0.13[d]
TADD - 11 - 3	14.50 ± 0.20[d]
TADD - 11 - 5	13.61 ± 0.03[d]
TADD - 15 - 1	21.38 ± 0.11[a]
TADD - 15 - 3	14.97 ± 0.03[cd]
TADD - 15 - 5	14.02 ± 0.12[d]
TADD - 20 - 1	13.78 ± 0.38[d]
TADD - 20 - 3	14.48 ± 0.01[d]
TADD - 20 - 5	17.63 ± 0.07[bc]
Bran	20.52 ± 0.49[ab]
Whole wheat	15.37 ± 0.08[cd]
Aleurone	15.20 ± 0.05[cd]

*Refer to Table 2 for sample abbreviations.

[abcd] Sample means within a column that have the same letter are not significantly different (α=0.05)

Table 4: Lipid content of wheat samples* [% (w / w), dry basis]

Sample	Lipid content
TADD - 11 - 1	3.67 ± 0.09^{cd}
TADD - 11 - 3	4.05 ± 0.04^{b}
TADD - 11 - 5	4.38 ± 0.03^{a}
TADD -15 - 1	3.81 ± 0.05^{bc}
TADD - 15 - 3	4.03 ± 0.05^{bc}
TADD - 15 - 5	4.04 ± 0.04^{b}
TADD - 20 - 1	3.94 ± 0.05^{bc}
TADD - 20 - 3	4.52 ± 0.09^{a}
TADD - 20 - 5	3.48 ± 0.06^{d}
Bran	2.92 ± 0.05^{e}
Whole wheat	1.38 ± 0.01^{f}
Aleurone	4.57 ± 0.03^{a}

*Refer to Table 2 for sample abbreviations.

^{abcd} Sample means within a column that have the same letter are not significantly different (α=0.05)

Table 5: Ash content of wheat samples* [% (w / w), dry basis]

Sample	Ash content
TADD - 11 - 1	4.05 ± 0.03[c]
TADD - 11 - 3	3.55 ± 0.02[d]
TADD - 11 - 5	3.37 ± 0.01[e]
TADD - 15 - 1	3.82 ± 0.04[c]
TADD - 15 - 3	3.581 ± 0.004[d]
TADD - 15 - 5	3.44 ± 0.01[e]
TADD - 20 - 1	4.52 ± 0.05[b]
TADD - 20 - 3	3.78 ± 0.01[c]
TADD - 20 - 5	3.21 ± 0.03[f]
Bran	4.05 ± 0.11[c]
Whole wheat	1.66 ± 0.01[g]
Aleurone	7.40 ± 0.03[a]

*Refer to Table 2 for sample abbreviations.

[abcd] Sample means within a column that have the same letter are not significantly different (α=0.05)

Table 6: Mineral compositions of wheat samples* [(mg / g), dry basis]

Sample	Mg	P	K	Ca
TADD - 11 - 1	2.89 ± 0.04	6.96 ± 0.05	10.16 ± 0.12	1.07 ± 0.03
TADD - 11 - 3	2.70 ± 0.02	6.49 ± 0.05	9.11 ± 0.10	0.80 ± 0.01
TADD - 11 - 5	2.63 ± 0.03	6.52 ± 0.07	8.39 ± 0.05	0.74 ± 0.01
TADD - 15 - 1	3.05 ± 0.07	6.22 ± 0.12	9.12 ± 0.29	1.44 ± 0.05
TADD - 15 - 3	2.84 ± 0.08	6.76 ± 0.21	9.24 ± 0.30	0.79 ± 0.05
TADD - 15 - 5	2.86 ± 0.03	6.94 ± 0.23	9.00 ± 0.18	0.83 ± 0.03
TADD - 20 - 1	3.35 ± 0.04	7.74 ± 0.10	11.53 ± 0.14	1.07 ± 0.01
TADD - 20 - 3	3.07 ± 0.05	7.60 ± 0.09	9.75 ± 0.19	0.85 ± 0.04
TADD - 20 - 5	2.73 ± 0.10	6.18 ± 0.25	8.07 ± 0.31	0.88 ± 0.05
Bran	3.74 ± 0.01	7.61 ± 0.02	8.23 ± 0.01	1.25 ± 0.002
Whole wheat	1.45 ± 0.03	3.21 ± 0.27	3.87 ± 0.28	0.52 ± 0.01
Aleurone	7.03 ± 0.08	16.09 ± 0.07	19.08 ± 0.12	1.03 ± 0.01

*Refer to Table 2 for sample abbreviations.

Table 7: Extraction yield [% (w / w), dry basis] and concentration (mg / mL) of wheat samples*

Sample	Concentration	Extraction yield
TADD - 11 - 1	10.13 ± 0.04	11.69 ± 0.04[ab]
TADD - 11 - 3	8.78 ± 0.28	9.03 ± 0.10[d]
TADD - 11 - 5	8.35 ± 0.26	9.49 ± 0.08[d]
TADD - 15 - 1	10.28 ± 0.50	12.83 ± 0.34[a]
TADD - 15 - 3	8.42 ± 0.35	10.50 ± 0.22[c]
TADD - 15 - 5	7.58 ± 0.09	8.12 ± 0.25[e]
TADD - 20 - 1	7.27 ± 0.24	6.97 ± 0.16[f]
TADD - 20 - 3	9.21 ± 0.22	11.59 ± 0.05[b]
TADD - 20 - 5	7.71 ± 0.03	10.32 ± 0.08[c]
Bran	10.11 ± 0.12	12.23 ± 0.03[ab]
Whole wheat	5.33 ± 0.28	7.28 ± 0.24[f]
Aleurone	11.32 ± 0.13	12.68 ± 0.10[a]

*Refer to Table 2 for sample abbreviations.

[abcd] Sample means within a column that have the same letter are not significantly different (α=0.05)

Table 8: Antioxidant capacity assay*.

Sample	**ORAC** (µmoles TE / g of sample, dry basis)	**ORAC** (µmoles TE / g of extract, dry basis)	**TPC** (GE mg / g sample, dry basis)	**TPC** (GE mg / g extract, dry basis)	**DPPH (% inhibition)**
TADD - 11 - 1	55.96 ± 0.85^{b}	478.69 ± 21.75	2.67 ± 0.01^{b}	22.81 ± 0.27	42.61 ± 0.43^{b}
TADD - 11 - 3	37.25 ± 0.56^{c}	415.55 ± 18.43	2.07 ± 0.03^{cd}	22.91 ± 1.00	25.96 ± 0.33^{f}
TADD - 11 - 5	26.86 ± 0.37^{e}	283.01 ± 11.63	2.37 ± 0.04^{c}	25.02 ± 0.92	40.21 ± 0.35^{c}
TADD - 15 - 1	32.92 ± 0.50^{cb}	256.56 ± 11.65	2.83 ± 0.05^{b}	22.06 ± 0.89	41.24 ± 0.42^{bc}
TADD - 15 - 3	29.66 ± 0.48^{de}	276.63 ± 12.84	2.77 ± 0.05^{b}	26.41 ± 1.12	40.79 ± 0.30^{bc}
TADD - 15 - 5	35.34 ± 0.54^{c}	435.19 ± 19.88	1.86 ± 0.02^{de}	22.88 ± 0.69	11.97 ± 0.17^{g}
TADD - 20 - 1	33.37 ± 0.44^{cd}	478.75 ± 18.78	1.78 ± 0.03^{e}	25.60 ± 1.23	23.60 ± 0.33^{f}
TADD - 20 - 3	54.79 ± 0.85^{b}	472.74 ± 21.97	2.67 ± 0.04^{b}	23.04 ± 0.76	33.01 ± 0.36^{d}
TADD - 20 - 5	$28.57 \pm 0.48d^{e}$	276.81 ± 13.84	2.70 ± 0.05^{b}	26.18 ± 1.09	13.00 ± 0.16^{g}
Bran	19.48 ± 0.31^{ef}	205.36 ± 9.63	2.41 ± 0.05^{bc}	19.66 ± 0.49	29.98 ± 0.32^{e}
Whole wheat	14.95 ± 0.23^{f}	159.27 ± 7.69	1.43 ± 0.01^{e}	19.68 ± 0.98	9.79 ± 0.14^{h}
Aleurone	52.19 ± 0.88^{b}	411.58 ± 20.86	2.79 ± 0.06^{b}	22.05 ± 1.08	42.43 ± 0.17^{b}
BHT	1234.22 ± 31.38^{a}	N/A	21.42 ± 0.41^{a}	N/A	56.99 ± 1.01^{a}

*Refer to Table 2 for sample abbreviations. N/A means not applicable.

[abcd] Sample means within a column that have the same letter are not significantly different (α=0.05)

Table 9: Tocols content and compositions of wheat samples* (μg / g sample, dry basis).

Sample	α - T+ α - T3**	β-T**	γ - T + γ - T3**	δ - T**	Total tocols content
TADD - 11 - 1	5.36 ± 0.21[b]	n.d.***	2.42 ± 0.07[b]	n.d.	7.78 ± 0.27[b]
TADD - 15 - 1	6.64 ± 0.21[a]	n.d.	3.82 ± 0.12[a]	n.d.	10.47 ± 0.31[a]
TADD - 15 - 3	4.43 ± 0.13[cd]	n.d.	2.23 ± 0.07[bc]	n.d.	6.66 ± 0.15[bc]
TADD - 20 - 3	7.70 ± 0.28[a]	n.d.	3.31 ± 0.14[a]	n.d.	11.02 ± 0.28[a]
TADD - 20 - 5	5.25 ± 0.15[bc]	n.d.	2.27 ± 0.09[bc]	n.d.	7.52 ± 0.14[b]
Bran	3.83 ± 0.05[d]	n.d.	1.94 ± 0.06[c]	n.d.	5.76 ± 0.08[c]
Whole wheat	3.02 ± 0.04[e]	n.d.	n.d.	n.d.	3.02 ± 0.04[d]
Aleurone	6.59 ± 0.20[a]	2.37 ± 0.09	1.54 ± 0.04[d]	1.70 ± 0.05	12.20 ± 0.35[a]

*Refer to Table 2 for sample abbreviations.

** α - T + α - T3: α - tocopherol + α- tocotrienol.
β - T: β - tocopherol.
γ - T + γ -T3: γ - tocopherol + γ - tocotrienol.
δ - T: δ - tocopherol.
*** n.d.: not detected.

[abcd] Sample means within a column that have the same letter are not significantly different

(α=0.05)

Table 10: Carotenoids content and compositions of wheat samples* (µg / g sample, dry basis).

Sample	Lutein	Zeaxanthin	Total Carotenoids content
TADD - 11 - 1	2.61 ± 0.02^{bc}	0.848 ± 0.004^{b}	3.46 ± 0.02^{cd}
TADD - 15 - 1	2.68 ± 0.02^{ab}	0.913 ± 0.005^{ab}	3.59 ± 0.02^{abc}
TADD - 15 - 3	2.84 ± 0.04^{ab}	0.95 ± 0.03^{a}	3.79 ± 0.06^{ab}
TADD - 20 - 3	2.92 ± 0.05^{a}	0.911 ± 0.007^{ab}	3.83 ± 0.05^{a}
TADD - 20 - 5	2.84 ± 0.03^{ab}	0.953 ± 0.005^{a}	3.79 ± 0.03^{ab}
Bran	2.630 ± 0.006^{b}	0.877 ± 0.003^{ab}	3.508 ± 0.007^{bcd}
Whole wheat	1.41 ± 0.01^{d}	0.480 ± 0.001^{c}	1.89 ± 0.01^{e}
Aleurone	2.40 ± 0.02^{c}	0.879 ± 0.007^{ab}	3.28 ± 0.03^{d}

*Refer to Table 2 for sample abbreviations.

^{abcd} Sample means within a column that have the same letter are not significantly different ($\alpha = 0.05$)

Table 11: Phenolic acids content and compositions of wheat samples* (µg / g sample, dry basis).

Sample	Benzoic acid	Vanillic acid	Caffeic Acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Total phenolic acids content
TADD - 11 - 1	6.13 ± 0.15^{cd}	5.76 ± 0.13^{d}	4.40 ± 0.10^{b}	4.73 ± 0.20^{c}	5.21 ± 0.17^{abcd}	14.98 ± 0.56^{a}	41.21 ± 0.88^{bc}
TADD - 15 - 1	6.23 ± 0.09^{cd}	5.81 ± 0.08^{d}	4.59 ± 0.05^{b}	4.42 ± 0.03^{c}	4.54 ± 0.03^{d}	8.58 ± 0.25^{c}	34.18 ± 0.30^{d}
TADD - 15 - 3	7.76 ± 0.17^{a}	8.30 ± 0.22^{b}	5.02 ± 0.08^{ab}	7.62 ± 0.26^{a}	5.80 ± 0.17^{ab}	10.50 ± 0.30^{bc}	45.00 ± 0.97^{ab}
TADD - 20 - 3	7.21 ± 0.16^{ab}	10.32 ± 0.20^{a}	4.90 ± 0.13^{ab}	7.14 ± 0.11^{ab}	6.12 ± 0.12^{a}	11.54 ± 0.26^{b}	47.23 ± 0.59^{a}
TADD - 20 - 5	6.54 ± 0.13^{bc}	6.89 ± 0.21^{c}	4.84 ± 0.10^{ab}	5.03 ± 0.10^{c}	5.55 ± 0.21^{abc}	8.66 ± 0.31^{c}	37.52 ± 0.76^{cd}
Bran	5.64 ± 0.18^{d}	6.24 ± 0.14^{cd}	4.81 ± 0.09^{ab}	7.16 ± 0.14^{ab}	4.84 ± 0.14^{bcd}	10.81 ± 0.47^{b}	39.51 ± 0.65^{c}
Whole wheat	3.37 ± 0.01^{e}	3.26 ± 0.02^{e}	n.d.**	2.53 ± 0.04^{d}	2.37 ± 0.12^{e}	4.80 ± 0.15^{d}	16.33 ± 0.12^{e}
Aleurone	6.41 ± 0.08^{bcd}	9.56 ± 0.32^{ab}	5.43 ± 0.19^{a}	6.20 ± 0.22^{b}	4.65 ± 0.15^{cd}	12.87 ± 0.22^{ab}	45.11 ± 0.93^{ab}

*Refer to Table 2 for sample abbreviations.

** n.d.: not detected

^{abcd} Sample means within a column that have the same letter are not significantly different ($\alpha = 0.05$)

Table 12: Organic acids content and compositions of wheat samples* (µmole / g sample, dry basis).

Sample	Citric acid	Ascorbic acid + Malic acid	Succinic acid	Fumaric acid	Total organic acids content
TADD - 11 - 1	17.89 ± 0.23^{b}	1.46 ± 0.03^{a}	24.96 ± 0.73^{d}	11.02 ± 0.17^{d}	55.33 ± 0.86^{c}
TADD - 15 - 1	25.18 ± 0.26^{a}	1.47 ± 0.04^{a}	37.72 ± 0.73^{a}	18.14 ± 0.22^{b}	82.50 ± 0.88^{a}
TADD - 15 - 3	22.67 ± 0.39^{a}	1.43 ± 0.05^{a}	21.16 ± 0.76^{e}	10.75 ± 0.19^{d}	56.01 ± 1.05^{bc}
TADD - 20 - 3	24.37 ± 0.42^{a}	1.14 ± 0.04^{b}	19.84 ± 0.58^{e}	10.85 ± 0.15^{d}	56.20 ± 0.81^{bc}
TADD - 20 - 5	18.25 ± 0.36^{b}	0.97 ± 0.03^{c}	28.76 ± 0.54^{cd}	13.96 ± 0.19^{c}	61.93 ± 0.72^{b}
Bran	23.22 ± 0.31^{a}	0.55 ± 0.01^{d}	32.01 ± 0.42^{bc}	20.87 ± 0.25^{a}	76.64 ± 0.48^{a}
Whole wheat	10.36 ± 0.15^{c}	0.0.308± 0.005^{f}	13.10 ± 0.29^{f}	6.09 ± 0.06^{e}	29.79 ± 0.40^{e}
Aleurone	1.08 ± 0.03^{d}	0.40 ± 0.01^{e}	34.12 ± 0.50^{ab}	11.00 ± 0.22^{d}	46.60 ± 0.61^{d}

*Refer to Table 2 for sample abbreviations.

[abcd] Sample means within a column that have the same letter are not significantly different

(α=0.05)

Table 13: Policosanol content and compositions of wheat samples* (µg / g sample, dry basis).

Sample	C20**	C21**	C22**	C23**	C24**	C26**	C27**	C28**	C30**	Total policosanol content
TADD - 11 - 1	2.90 ± 0.14^{ab}	0.73 ± 0.03^{a}	2.04 ± 0.01^{d}	5.63 ± 0.17^{b}	8.94 ± 0.37^{b}	12.56 ± 0.26^{b}	1.79 ± 0.06^{c}	13.95 ± 0.41^{b}	9.55 ± 0.21^{c}	58.09 ± 0.78^{b}
TADD - 15 - 1	3.24 ± 0.05^{a}	0.49 ± 0.04^{bc}	3.12 ± 0.08^{a}	6.71 ± 0.36^{a}	10.07 ± 0.37^{b}	10.74 ± 0.44^{c}	1.51 ± 0.09^{cd}	10.61 ± 0.32^{c}	9.56 ± 0.25^{c}	56.05 ± 0.88^{bc}
TADD - 15 - 3	2.32 ± 0.14^{c}	0.39 ± 0.03^{c}	1.37 ± 0.09^{e}	4.50 ± 0.28^{c}	12.60 ± 0.66^{a}	8.77 ± 0.39^{d}	2.26 ± 0.07^{a}	8.69 ± 0.25^{d}	6.44 ± 0.22^{d}	47.35 ± 1.67^{e}
TADD - 20 - 3	2.51 ± 0.10^{bc}	0.39 ± 0.02^{c}	2.59 ± 0.12^{bc}	5.40 ± 0.16^{bc}	8.40 ± 0.30^{b}	9.19 ± 0.26^{d}	2.22 ± 0.11^{ab}	9.68 ± 0.31^{cd}	11.23 ± 0.42^{b}	51.62 ± 0.77^{d}
TADD - 20 - 5	2.48 ± 0.08^{bc}	0.181 ± 0.006^{d}	2.77 ± 0.12^{ab}	4.62 ± 0.27^{bc}	4.55 ± 0.17^{c}	5.44 ± 0.28^{e}	1.30 ± 0.10^{d}	6.23 ± 0.10^{e}	8.23 ± 0.31^{c}	35.80 ± 0.36^{f}
Bran	2.76 ± 0.10^{bc}	0.55 ± 0.04^{b}	2.95 ± 0.12^{ab}	5.38 ± 0.17^{bc}	9.34 ± 0.37^{b}	9.81 ± 0.29^{cd}	1.56 ± 0.09^{cd}	10.69 ± 0.37^{c}	9.33 ± 0.24^{c}	52.39 ± 0.94^{cd}
Whole wheat	1.17 ± 0.02^{d}	0.48 ± 0.03^{bc}	1.03 ± 0.06^{e}	4.50 ± 0.18^{c}	5.89 ± 0.16^{c}	3.14 ± 0.14^{f}	0.31 ± 0.01^{e}	1.40 ± 0.09^{f}	3.77 ± 0.18^{e}	21.70 ± 0.14^{g}
Aleurone	2.82 ± 0.12^{ab}	0.12 ± 0.01^{d}	2.26 ± 0.06^{cd}	0.65 ± 0.04^{d}	4.73 ± 0.24^{c}	23.11 ± 0.45^{a}	1.85 ± 0.09^{bc}	15.89 ± 0.73^{a}	13.38 ± 0.61^{a}	64.81 ± 0.53^{a}

*Refer to Table 2 for sample abbreviations.

**Eicosanol (C20), heneicosanol (C21), docosanol (C22), tricosanol (C23), tetracosanol (C24), hexacosanol (C26), heptacosanol (C27), octacosanol (C28), and triacontanol (C30)

[abcd] Sample means within a column that have the same letter are not significantly different (α=0.05)

Table 14: Phytosterol content and compositions of wheat samples* (µg / g sample, dry basis).

Sample	β - Sitosterol	Stigmasterol	Campesterol	Total phytosterol content
TADD - 11 - 1	1329.77 ± 21.59[a]	59.86 ± 1.10[e]	270.55 ± 3.83[bc]	1660.18 ± 26.47[a]
TADD - 15 - 1	804.26 ± 20.17[c]	101.82 ± 2.46[b]	281.52 ± 8.21[b]	1187.60 ± 30.81[c]
TADD - 15 - 3	1170.65 ± 53.10[b]	56.11 ± 0.84[e]	268.17 ± 4.44[bc]	1494.93 ± 49.69[b]
TADD - 20 - 3	636.75 ± 10.30[d]	94.34 ± 0.79[c]	217.69 ± 5.15[d]	948.78 ± 9.23[d]
TADD - 20 - 5	341.10 ± 4.17[e]	46.64 ± 0.32[f]	115.4 ± 1.21[e]	503.16 ± 5.67[e]
Bran	638.35 ± 10.12[d]	77.5 ± 1.01[d]	257.3 ± 2.26[c]	973.20 ± 13.30[d]
Whole wheat	252.56 ± 11.66[e]	10.43 ± 0.24[g]	57.94 ± 1.03[f]	320.93 ± 11.78[f]
Aleurone	702.93 ± 21.31[cd]	158.96 ± 3.04[a]	407.18 ± 3.48[a]	1269.07 ± 27.72[c]

*Refer to Table 2 for sample abbreviations.

[abcd] Sample means within a column that have the same letter are not significantly different (α=0.05)

Table 15: Contribution of bioactive compounds to ORAC assay (r^2)

Compounds	r^2
Ferulic acid	0.6282
α-Tocopherol + α-Tocotrienol	0.5904
Vanillic acid	0.3673
***p*-Coumaric acid**	0.2693
Benzoic acid	0.2533
Caffeic acid	0.2369
γ-Tocopherol + γ-Tocotrienol	0.2342
Lutein	0.1857
Zeaxanthin	0.1635
β-Tocopherol	0.1615
δ-Tocopherol	0.1573
Ascorbic acid + Malic acid	0.1269
Syringic acid	0.0866
Fumaric acid	0.0397
Citric acid	0.0177
Succinic acid	0.0167

Table 16: Contribution of bioactive compounds to DPPH assay (r^2)

Compounds	r^2
Ferulic acid	0.5497
Caffeic acid	0.4293
Benzoicvacid	0.354
Zeaxanthin	0.2986
Ascorbic acid + Malic acid	0.2874
Lutein	0.2718
Syringic acid	0.2513
α-Tocopherol + α-Tocotrienol	0.2402
Vanillic acid	0.2156
***p*-Coumaric acid**	0.2136
Succinic acid	0.2093
δ-Tocopherol	0.1000
β-Tocopherol	0.0995
Fumaric acid	0.0602
γ-Tocopherol + γ-Tocotrienol	0.0197
Citric acid	0.0161

Table 17: Contribution of bioactive compounds to TPC assay (r^2)

Compounds	r^2
Caffeic acid	0.8750
Benzoic acid	0.7008
***p*-Coumaric acid**	0.5851
Vanillic acid	0.4197
Ferulic acid	0.3800
Syringic acid	0.3404

Figure 1: A typical ORAC assay kinetic curve (Adapted from Cao 1993)

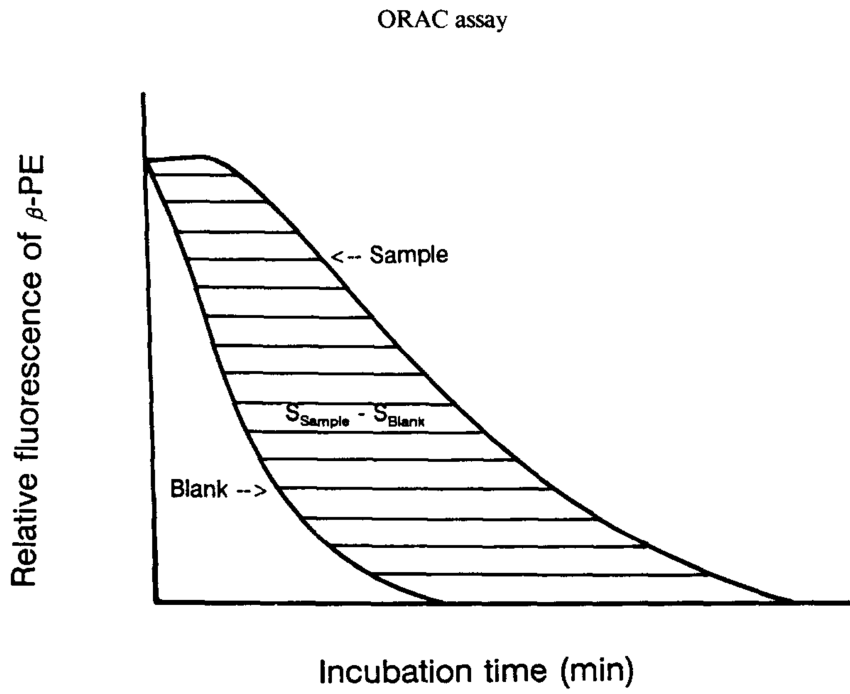


Figure 2: Wheat kernel structure (Adapted from Yu 2008)

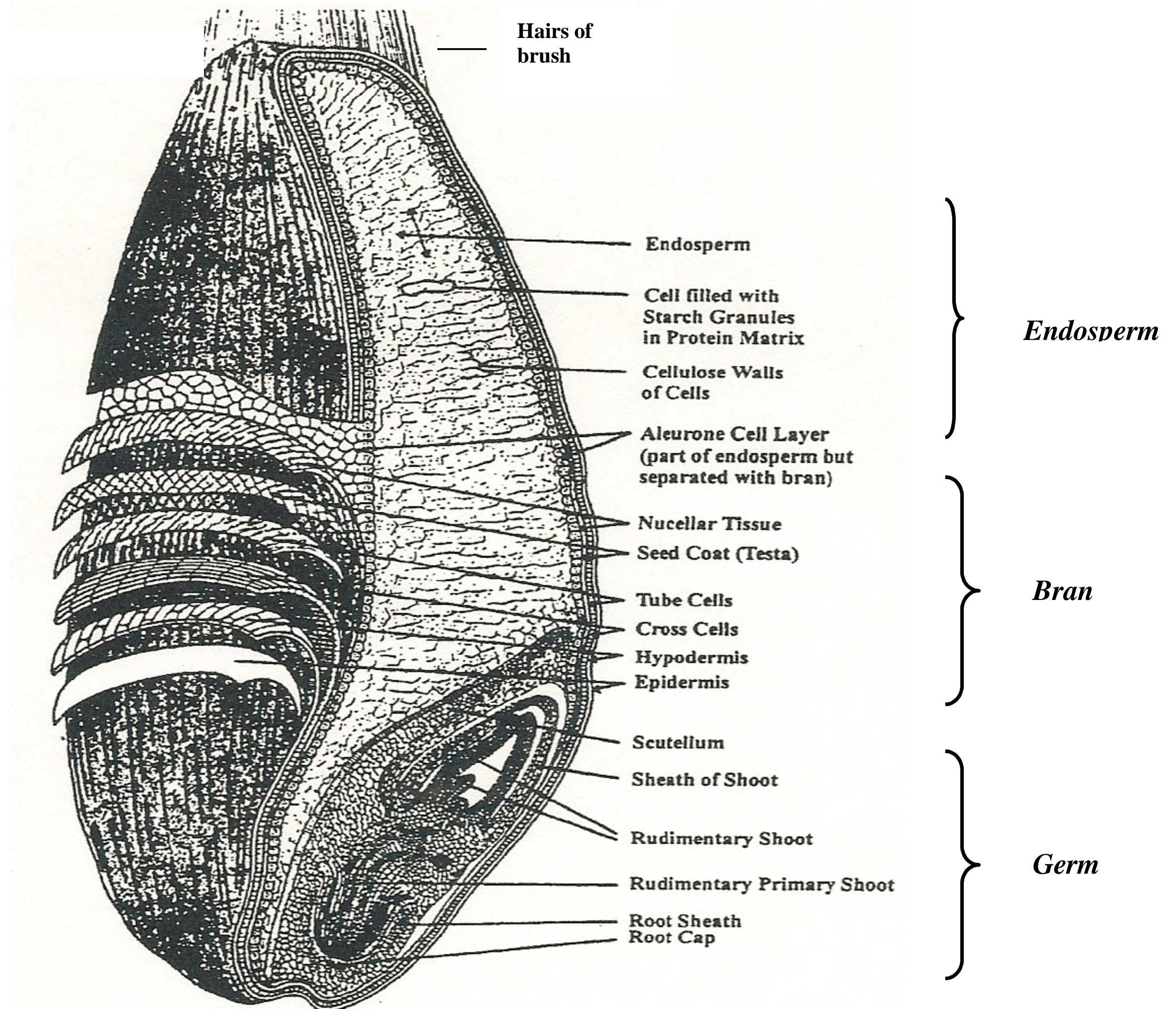


Figure 3: Roller mill

Figure 4: Schematic view of the internal flow of stock in a debranner (Adapted from Dexter and Wood 1996)

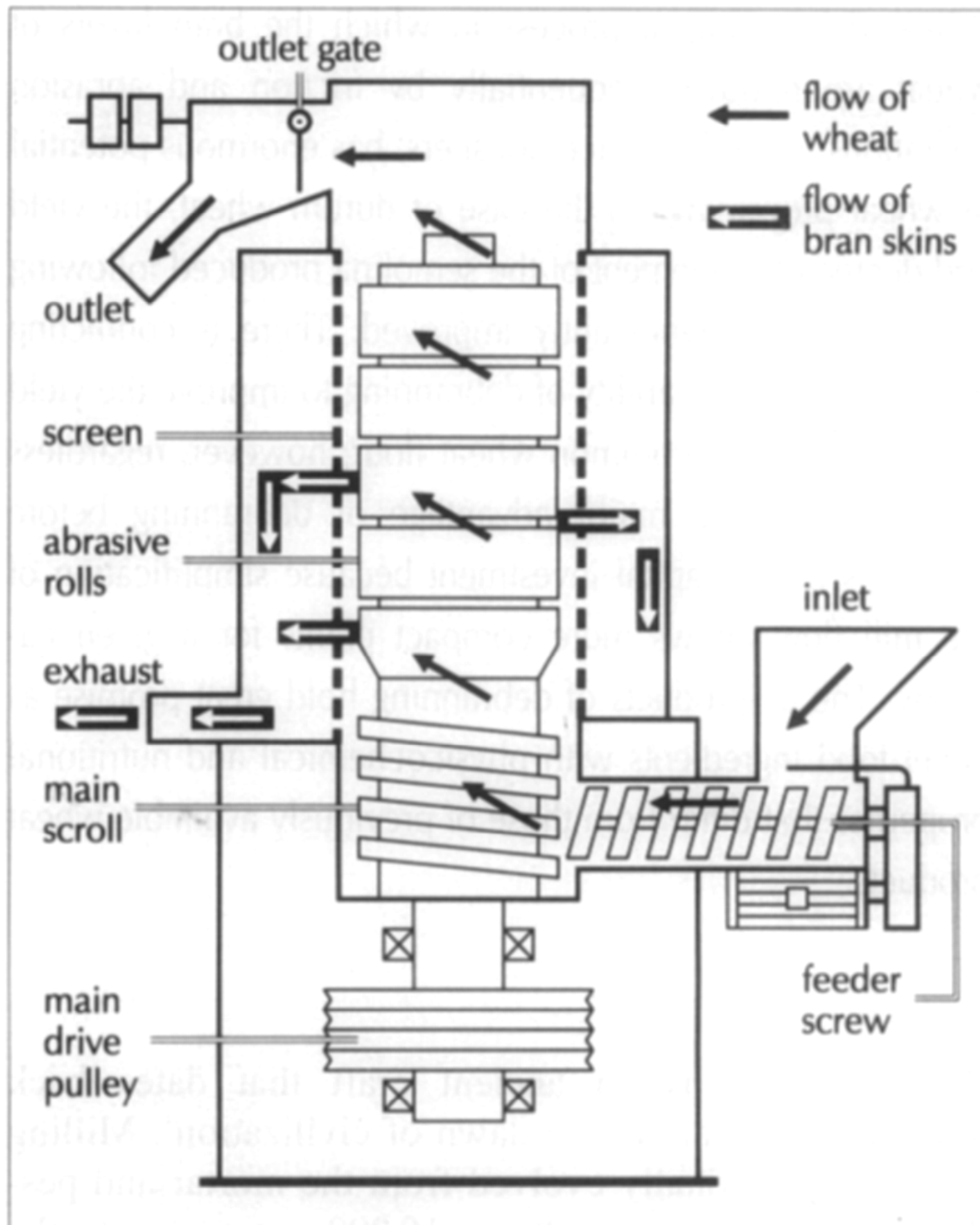


Figure 5: Self-abrasive plate of TADD

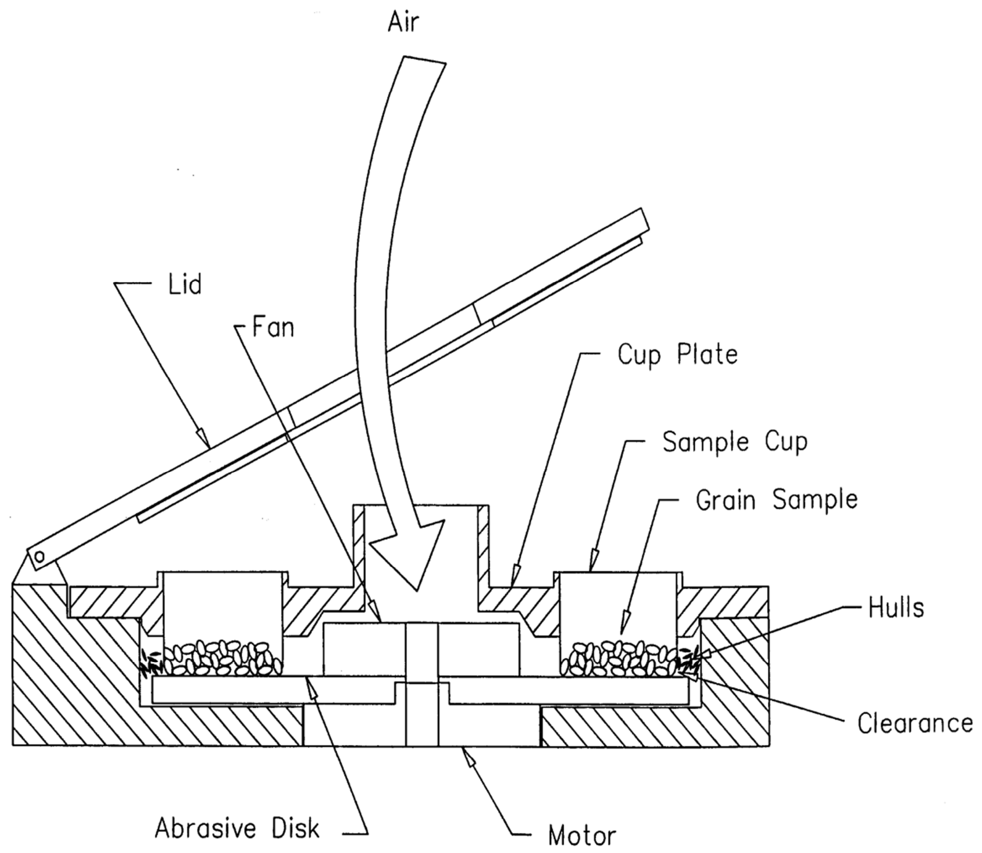


Figure 6: Bran collection device of TADD

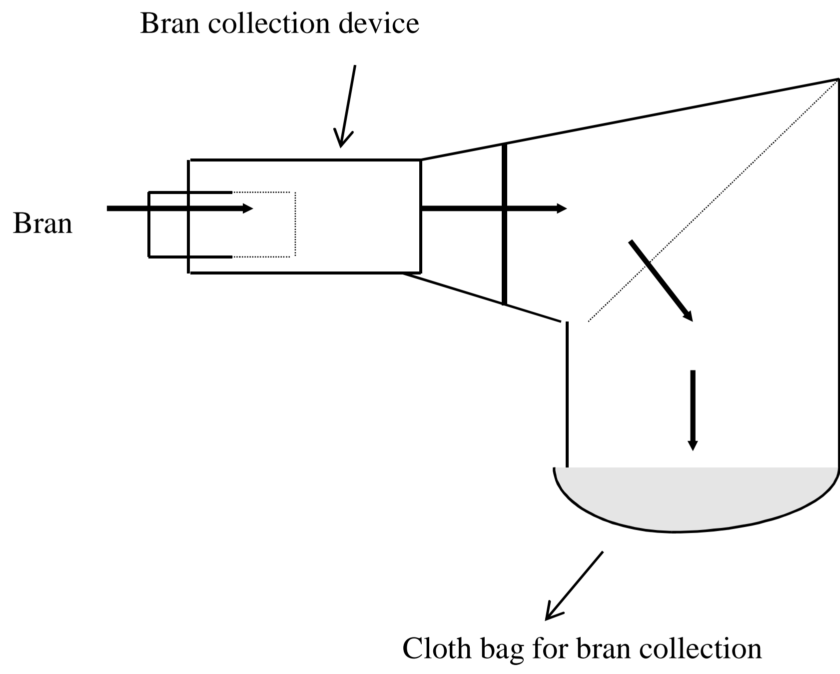
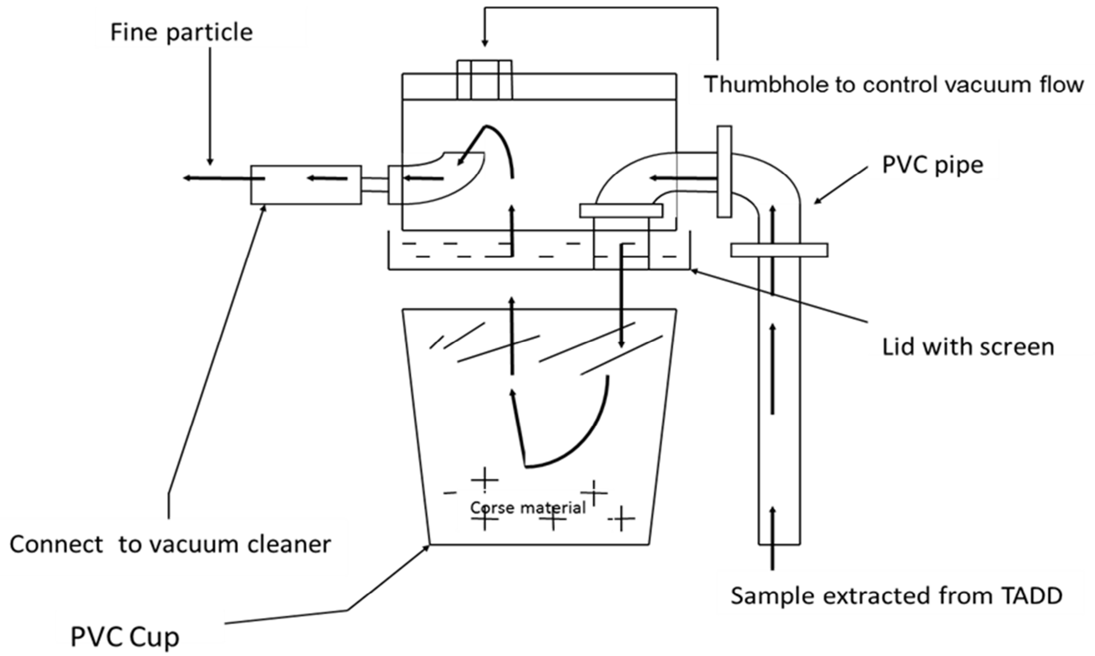


Figure 7: Vacuum aspirating device of TADD



VITA

YONGFEN CHEN

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFICIENCY OF ABRASIVE DEHULLING TO PRODUCE WHEAT GRAIN FRACTIONS ENRICHED IN ANTIOXIDANTS

Major Field: Food Science

Biographical:

Personal Data: Born in Urumuqi, Xinjiang, China, on July 11, 1981, the daughter of Mr. Jiyan Chen and Mrs. Shuzhen Liu.

Education: Completed the requirements for the Doctor of Philosophy in Food at Oklahoma State University, Stillwater, Oklahoma in May, 2011.

Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in December, 2007.

Completed the requirements for the Bachelor of Science in Food Science at China Agricultural University, Beijing, China in June, 2004.

Experience: Employed by Oklahoma State University, Department of Plant and Soil Science as a graduate research assistant; Oklahoma State University, Department of Plant and Soil Sciences, 2005 to 2007.Employed by Oklahoma State University, Department of Biosystem and Agricultural Engineering as graduate research assistant; Oklahoma State University, Department of Biosystem and Agricultural Engineering, 2007 to 2010.

Professional Memberships: Institute of Food Technologists, American Oil Chemists' Society

Name: Yongfen Chen Date of Degree: May, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EFFICIENCY OF ABRASIVE DEHULLING TO PRODUCE WHEAT GRAIN FRACTIONS ENRICHED IN ANTIOXIDANTS

Pages in Study: 120 Candidate for the Degree of Doctor of Philosophy

Major Field: Food Science

Scope and Method of Study: The main objective of this study was to examine the efficiency of the tangential abrasive dehulling method to produce wheat fractions enriched in health beneficial bioactive compounds. Nine wheat fractions obtained by using a tangential abrasive dehulling device (TADD) at three different abrasion time and grain moisture levels were analyzed for their chemical composition. Aleurone, bran and whole wheat were also examined as references. Antioxidant capacity of the extracts obtained from the samples was evaluated by using ORAC, TPC and DPPH assay. Tocols, carotenoids, phenolic acids and organic acids content and compositions were determined by using various HPLC methods. Total policosanol and phytosterol content together with composition in whole wheat grain were determined by GC-FID. Protein, ash, starch, lipids, TADD yield, extraction yield and mineral content were also analyzed. The correlations between bioactive compounds and the antioxidant capacity of the extracts were derived.

Findings and Conclusions: Most TADD samples showed stronger antioxidant capacity and greater bioactive compounds content than that of the bran and whole wheat samples. We were able to obtain wheat fractions that contained higher amount of bioactive compounds than the commercial aleurone product. Both moisture andabrasive time had significant effect on the antioxidant capacity of the samples. Shorter abrasive time and moderate grain moisture content resulted in fractions enriched in bioactive compounds. Ferulic acid played the most important role in the ORAC and DPPH assays. The results indicated that TADD is an efficient method to enrich bioactive compounds in wheat fractions.

ADVISER'S APPROVAL: Nurhan. T. Dunford