EXTRUSION OF WHEAT WASHED BRAN: PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES

By

George Nyombaire

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ABSTRACT

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Milled wheat bran contains substantial amounts of residual starchy endosperm that may interfere with the analyses that are used to determine a bran's composition and physicochemical properties. In addition, it is possible that during extrusion cooking, this residual starchy endosperm may undergo certain chemical modifications that may affect composition, physicochemical properties, and functionality of wheat bran. Therefore, objectives of the present study were: (1) to develop a method that removes most of the residual starchy endosperm still adherent to milled wheat bran, (2) to investigate the effects of particle size of non-washed and washed wheat bran coupled with extrusion processing conditions on total dietary fiber, insoluble dietary fiber, and soluble dietary fiber contents of non-washed and washed bran, (3) to investigate the effects of extrusion cooking variables on the physicochemical properties of extruded wheat bran, and (4) to evaluate the effects of treated wheat bran on the baking properties of bread and cookies.

The washing method developed in the present study reduced starch adherent to milled wheat bran by 76% (w/w), changed insoluble dietary fiber content in the bran from 39% to 69% (w/w), and decreased soluble fiber content from 4.93% in NWB to 1.68% (w/w). The water binding capacity was higher for washed bran and was not affected by bran particle size.

To investigate the effects of particle size coupled with extrusion processing conditions, non-washed bran (NWB), ground to pass through 1000 μm (NWB1000) and 425 μm (NWB425)

screens, and washed bran (WB), ground to pass through 1000 µm (WB1000) and 425 µm (WB425) screens, were extruded through a co-rotating and inter-meshing twin-screw extruder under conditions of varying screw configurations (low and high shear), feed moisture (25 and 35%), screw speed (100 and 400 rpm), and die temperature (100 and 150^{O}C). Extrusion increased soluble dietary fiber in both NWB1000 and NWB425 but decreased insoluble dietary fiber. Certain extrusion conditions increased or decreased the contents of insoluble and soluble fiber in WB425. The insoluble dietary fiber contents were lower in extrudates made from WB1000 than in non-extruded WB1000. Extrusion conditions increased or decreased soluble dietary fiber in WB1000.

The effects of extrusion processing on the physicochemical properties of wheat bran samples were studied. Extrusion increased the water binding capacity (WBC) of NWB samples. Washing and extrusion decreased weight average molecular weight of soluble dietary fibers extracted from NWB and WB samples. Differential Scanning Calorimetry results indicated that thermal decomposition properties of extrudates made from NWB and WB samples were not affected by extrusion. *In vitro* binding of bile acids was highest in extrudates made from WB1000, whereas the lowest binding of bile acids was in extrudates made from NWB425.

Baking studies indicated that bread formulations containing wheat bran resulted in breads with decreased loaf volumes. Substituting soft wheat flour with 5% or 15% of the prepared bran samples significantly decreased cookie spread. The contents of insoluble dietary fiber in the crumb and crusts of breads baked from hard wheat flour and hard wheat flour substituted with bran were higher than those in the unbaked flours. The contents of soluble dietary fiber were not significantly different from each other in the crumb and crusts of breads baked from flour samples substituted with bran.

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TABLE OF CONTENTS

LIST OF TABLES ix

LIST OF FIGURES xi

CHAPTER 1
INTRODUCTION 1
LITERATURE CITED 6

CHAPTER 2
LITERATURE REVIEW 9
2.1. WHEAT BRAN 10
2.2. DIETARY FIBER 14
2.2.1. Insoluble Dietary Fiber 16
2.2.1.1. Cellulose 16
2.2.1.2. Hemicelluloses 17
2.2.1.3. Lignin 18
2.2.2. Soluble Dietary Fiber 18
2.2.2.1. Arabinoxylans 19
2.2.2.2. β-glucans 20
2.2.2.3. Pectin 22
2.2.3. Non-Digestible Oligosaccharides 22
2.2.4. Resistant Starch 23
2.2.5. History of Dietary Fiber 24
2.2.5. Health Benefits of Dietary Fiber 25
2.3. PHYSICOCHEMICAL PROPERTIES OF HIGH FIBER INGREDIENTS 27
2.3.1. Solubility 27
2.3.2. Water Binding Capacity 28
2.3.3. Bile Acid Binding Capacity 28
2.3.4. Molecular Weight Distribution 29
2.3.5. Thermal Analysis 30
2.4. EFFECT OF PROCESSING ON DIETARY FIBER 32
2.4.1. Particle Size Distribution 32
2.4.2. Extrusion Cooking 33
2.5. WHEAT FLOUR 36
2.6. BAKED PRODUCTS 38
LITERATURE CITED 40

CHAPTER 3
PHYSICOCHEMICAL PROPERTIES OF WASHED WHEAT BRAN 49
3.1. ABSTRACT 50
3.2. INTRODUCTION 51
3.3. MATERIALS AND METHODS 53
3.3.1. Wheat Bran Sample 53

3.3.2. Washing of wheat bran 53
3.3.3. Determination of Total Starch 56
3.3.4. Thermal Properties of Non-Washed and Washed Wheat Bran Samples 57
3.3.5. Water Binding Capacity of Non-Washed and Washed Bran 57
3.3.6. Determination of Insoluble, Soluble, and Total Dietary Fiber Content 58
3.3.7. Proximate Compositions of Non-Washed and Washed Bran 60
3.3.8. Statistical Analysis 60
3.4. RESULTS AND DISCUSSION 61
3.4.1. Total Starch 61
3.4.2. Effect of Washing on the Thermal Properties of Non-Washed and Washed Wheat Bran Starch Samples 63
3.4.3. Water Binding Capacity of Non-Washed and Washed Bran Samples 66
3.4.4. Insoluble Dietary Fiber, Soluble Dietary Fiber and Total Dietary Fiber 68
3.4.5. Proximate Compositions (dry weight basis) of Non-Washed and Washed Wheat Bran 70
3.5. CONCLUSIONS 72
LITERATURE CITED 73

CHAPTER 4
EFFECTS OF PARTICLE SIZE COUPLED WITH EXTRUSION PROCESSING CONDITIONS ON DIETARY FIBER PROFILE OF NON-WASHED AND WASHED WHEAT BRAN 77
4.1. ABSTRACT 78
4.2. INTRODUCTION 79
4.3. MATERIALS AND METHODS 81
4.3.1. Wheat Bran Sample 81
4.3.2. Preparation of Washed Wheat Bran 81
4.3.3. Grinding and Sifting 81
4.3.4. Extrusion of Bran Samples 81
4.3.5. Determination of Soluble Dietary Fiber and Insoluble Dietary Fiber in Non-Washed and Washed Wheat Bran 86
4.3.7. Statistical Analysis 86
4.4. RESULTS AND DISCUSSION 87
4.4.1. Effect of Bran Particle Size Coupled with Extrusion Processing Conditions Under Low Shear Screw Configuration on the Contents of Insoluble Dietary Fiber (dry weight basis) in Extrudates Made from Non-Washed and Washed Wheat Bran 87
4.4.2. Effect of Bran Particle Size and Extrusion Processing Conditions Under Low Shear Screw Configuration on the Contents of Soluble Dietary Fiber (dry weight basis) in Extrudates Made from Non-Washed and Washed Wheat Bran 91
4.4.3. Effect of Bran Particle Size and Extrusion Processing Conditions Under Low and High Shear Screw Configurations on the Contents of Insoluble and Soluble Dietary Fibers in Extrudates Made from WB425 and WB1000 95
4.5. CONCLUSIONS 98
LITERATURE CITED 99

CHAPTER 5

EFFECTS OF EXTRUSION PROCESSING ON THE PHYSICOCHEMICAL PROPERTIES OF NON-WASHED AND WASHED WHEAT BRAN 103
5.1. ABSTRACT 104
5.2. INTRODUCTION 105
5.3. MATERIALS AND METHODS 108
5.3.1. Raw Material 108
5.3.2. Preparation of Washed Wheat Bran 108
5.3.3. Grinding and Sifting of Wheat Bran Samples 108
5.3.4. Extrusion of Wheat Bran 108
5.3.5. *In vitro* Binding of Bile Acids 108
5.3.6. Determination of Weight-Average Molecular Weight of Soluble Fiber Using High-Performance Size Exclusion Liquid Chromatography 109
5.3.7. Thermal Decomposition Properties of Wheat Bran Samples 110
5.3.8. Water Binding Capacity 110
5.3.9. Statistical Analysis 111
5.4. RESULTS AND DISCUSSION 112
5.4.1. Water Binding Capacity 112
5.4.2. Effect of Washing, Bran Particle Size and Extrusion on Weight-Average Molecular Weights of Soluble Fibers Isolated from Wheat Bran Samples 115
5.4.3. Thermal Decomposition of Wheat Bran Samples 117
5.4.4. Binding of Bile Acids by Wheat Bran Samples 119
5.5. CONCLUSIONS 122
LITERATURE CITED 123

CHAPTER 6
EFFECT OF TREATED WHEAT BRAN ON THE BAKING PROPERTIES OF BREAD AND COOKIES 128
6.1. ABSTRACT 129
6.2. INTRODUCTION 130
6.3. MATERIALS AND METHODS 132
6.3.1. Materials 132
6.3.2. Preparation of Washed Bran 132
6.3.3. Grinding and Sifting 132
6.3.4. Preparation of Extruded Wheat Bran 132
6.3.5. Farinograph Properties 132
6.3.6. Pasting Properties 133
6.3.7. Breadmaking 133
6.3.8. Determination of Bread Quality Properties 134
6.3.9. Cookiemaking 134
6.3.10. Determination of Cookie Quality Properties 135
6.3.11. Determination of Insoluble and Soluble Dietary Fibers in Bread Crumb and Crust 135
6.3.12. Statistical analysis 136
6.4. RESULTS AND DISCUSSION 137
6.4.1. Farinograph 137
6.4.2. Pasting Properties 140
6.4.3. Bread Quality Properties 142

6.4.4. Cookie Quality Properties 145
6.4.5. Effect of Baking on the Insoluble and Soluble Dietary Fibers in Bread 147
6.5. CONCLUSIONS 151
LITERATURE CITED 152

CHAPTER 7
GENERAL CONCLUSIONS 156

CHAPTER 8
FUTURE RECOMMENDATIONS 163

LIST OF TABLES

Table 2.1. Vitamin Contents (μg/g dry basis) of Hard Red Spring Wheat and its Milling Fractions 12

Table 2.2. Effect of Fiber Supplements on Fecal Bulk 13

Table 2.3. Constituents of Dietary Fiber 15

Table 2.4. β-Glucan Content (dry weight basis) of Selected Cereal Grains 21

Table 3.1. Total Starch Composition of Non-Washed and Washed Wheat Bran Samples 62

Table 3.2. Effect of Washing on the Thermal Properties of Non-Washed and Washed 65

Table 3.3. Water Binding Capacity (WBC) of Non-Washed and Washed Wheat Bran 67

Table 3.4. Dietary Fiber Composition of Non-Washed and Washed Wheat Bran samples 69

Table 3.5. Proximate Composition of Non-Washed and Washed Bran Samples 71

Table 4.1. Experimental Design 83

Table 4.2. Screw Elements Used for Low and High Shear Extrusion Configurations 84

Table 4.3. Extrusion Cooking Conditions for NWB425, NWB1000, WB425, and WB1000 85

Table 4.4. Effects of Extrusion Cooking Conditions under Low Shear Screw Configuration on the Contents of Insoluble and Soluble Dietary Fibers of Extrudates Made from NWB425 and NWB1000 Samples 90

Table 4.5. Effects of Extrusion Processing Conditions under Low Shear Screw Configuration on the Contents of Insoluble and Soluble Dietary Fibers of Extrudates Made from WB425 and WB1000 Samples 94

Table 5.1. Effects of Extrusion Conditions under Low Shear Screw Configuration on the Water Binding Capacities of NWB425, NWB1000, WB425, and WB1000 Samples 114

Table 5.2. Effect of Washing, Particle Size, and Extrusion on the Weigh Average Molecular Weight of Soluble Dietary Fiber in Wheat Bran Samples 116

Table 5.3. Thermal Decomposition Properties of Non-Washed Bran, Washed Bran, Extruded Non-Washed Bran, and Extruded Washed Bran Samples 118

Table 5.4. *In Vitro* Binding of Bile Acids by Wheat Bran Samples 121

Table 6.1. Farinograph Properties of Hard Wheat Flour Substituted with Bran 139

Table 6.2. Pasting Properties of Hard Wheat Flour and Soft Wheat Flour Substituted with Wheat Bran..... 141

Table 6.3. Quality Properties of Bread 144

Table 6.4. Effect of Bran Substitution on Quality Properties of Cookies..... 146

LIST OF FIGURES

Figure 3.1. Modified servodyne mixer used to wash wheat bran. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation. 55

Figure 4.1. Effects of low and high shear screw configurations (see Table 4.2 for details) on the contents of soluble dietary fiber in washed wheat bran extrudates. 96

Figure 4.2. Effect of low and high shear screw configurations (see Table 4.2 for details) on the contents of insoluble dietary fiber in WB1000 extrudates..... 97

Figure 6.6. Insoluble dietary fiber in flour samples and its quantity and distribution in subsequent baked bread. 149

Figure 6.7. Insoluble dietary fiber in flour samples and its quantity and distribution in subsequent baked bread. 150

CHAPTER 1

INTRODUCTION

Wheat is one of the most cultivated and consumed food plants in the world after corn and rice. The world annual production of wheat grain is approximately 600 million metric tons (Wrigley 2009). Wheat is considered a unique cereal because it contains gluten proteins that when hydrated can form dough with viscoelastic properties. This uniqueness is the main reason why wheat, especially in the flour form, is used to manufacture bread, which is by far one of the most common yeast-leavened products. Consumers prefer the texture of wheat-based products to any other cereal product (Hoseney 1998).

Wheat bran is a by-product of roller milling during the milling process of wheat grain. Wheat bran, which used to be exclusively used as animal feed, is now used by the baking industry as a major source of dietary fiber (Vetter 1988). Wheat bran is rich in dietary fiber (USDA 2011).

In the mid-1970s, scientists hypothesized that consumption of indigestible polysaccharides (high fiber ingredients) may prevent chronic diseases including heart disease and certain types of cancers. Since then, sufficient research data on the effects of dietary fiber in human health have supported the hypothesis. Because of the nutritional benefits of dietary fiber, health care officials and nutritionists have recommended that consumption of dietary fiber can help to maintain good health (McCleary 2007). Dietary fiber reduces the risk of cardiovascular disease, certain forms of cancer, and constipation (Schaafsma 2004). Dietary fibers are categorized as insoluble and soluble dietary fibers and each plays a different role in human health. Insoluble dietary fiber is important for proper bowel function and may reduce symptoms of chronic constipation, diverticular disease, and hemorrhoids, while soluble dietary fiber is associated with reduction in cholesterol levels and attenuation of blood glucose (Schaafsma 2004). The physiological functions of dietary fibers are related to their physicochemical properties such as water holding capacity, viscosity and susceptibility to bacterial degradation in the colon (Dikeman and Fahey 2006). Because of the

health benefits of dietary fiber, the food industry is developing fiber-enriched food products, including dairy products, but bakery products remain the most preferred source of dietary fiber (Hamid and Luan 2000; Sanchez-Alonso et al 2007; Collar 2008). However, incorporation of fiber during food processing alters the consistency, texture, rheological properties and sensory attributes of fiber-enriched food products (Collar et al 2009). For example, addition of fiber during the bread-making process affects the mixing properties and rheological behavior of the dough due to the interaction of fiber ingredients with starch and protein in the bread flour (Rosell and Foegeding 2007). Incorporation of high fiber ingredients results in loaves with reduced volume, cookies with reduced spread and hard texture; and reduces expansion of extruded products (Pomeranz et al 1977; Lai et al 1989; Dreher 1987; Zang and Moore 1999). The challenge for the food industry is to develop better methods to produce fiber-enriched food products with acceptable quality.

It is important to understand that the amount of soluble dietary fiber is lower than that of insoluble dietary fiber in wheat bran, which implies that most of the health benefits provided by wheat bran such as proper bowel function are attributable to insoluble dietary fiber. The amount of soluble dietary fiber in wheat bran is 1.5 to 4.0% (w/w) whereas insoluble dietary fiber is 35 to 48% (w/w) (Kahol and Chow 2000; Nandini and Silimath 2001; Esposito et al 2005). The ability of soluble dietary fiber to lower serum cholesterol and attenuate blood glucose has prompted scientists to find a way of increasing soluble dietary fiber in wheat bran. Some investigators have used extrusion cooking technology to increase the content of soluble dietary fiber in native (non-treated) wheat bran.

It is very important to note that although the major objective of roller milling is to separate wheat bran as cleanly as possible from the adherent starchy endosperm, it is almost impossible to

produce pure bran during the milling process. In certain cases, some investigators (Caprez et al 1986; Camire et al 1997) have concluded that extrusion cooking causes mechanical rupture of insoluble dietary fiber leading to an increase in soluble dietary fiber content. However, it is crucial to investigate the role of residual starchy endosperm still adherent to bran after the milling process on physicochemical properties and dietary fiber profile of that wheat bran. Also, it is important to look at the role of the residual starchy endosperm on the physicochemical properties and dietary fiber profile of extruded wheat bran. Therefore to obtain reliable dietary fiber results, it is important to conduct extrusion cooking experiments using wheat bran that has been treated to remove most of the starch adherent to the bran. Starch is known to undergo chemical modifications during extrusion processing that may interfere with dietary fiber analyses (Fogliano et al 1999).

Therefore, the present study about extrusion of washed wheat bran was designed with the following objectives:

1. To develop a method that removes most of the residual starchy endosperm still adherent to milled wheat bran.
2. To investigate the effects of particle size of non-washed and washed wheat bran coupled with extrusion processing variables on total dietary fiber, insoluble dietary fiber, and soluble dietary fiber contents.
3. To investigate the effects of extrusion cooking variables on physicochemical properties of extruded wheat bran.
4. To investigate the effects of extrusion processing variables on *in vitro* binding of bile acids by extruded washed bran.

5. To evaluate the effects ground extruded washed bran on the baking properties of bread and cookies.

The purpose of the present research is to provide scientific information on how the residual starchy endosperm still adherent to wheat bran after the process of roller-milling affects its physicochemical properties and dietary fiber composition. The following dissertation is divided into: (1) Literature review, (2) Physicochemical properties of washed wheat bran, (3) Effects of particle size coupled with extrusion processing conditions on dietary fiber profile of non-washed and washed wheat bran, (4) Effects of extrusion processing on physicochemical properties of washed wheat bran, (5) Effect of treated wheat bran on the baking properties of bread and cookies, (6) General conclusions, and (7) Recommendations for future studies. The chapters of this dissertation were written according to the Cereal Chemistry journal article format and thus some information such as introduction and laboratory procedures are very similar for certain chapters.

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CHAPTER 2

LITERATURE REVIEW

2.1. WHEAT BRAN

World wheat production is approximately 685 million metric tons per year resulting in nearly 140 million metric tons of wheat bran (World Agricultural Outlook Board 2012). Wheat bran is the outer coat of the wheat kernel and comprises about 12-15% (w/w) of wheat grain (Cho and Clark 2001). It is separated from wheat during the process of wheat milling. Wheat brans from different wheat varieties, for example, red, white, soft, hard, and durum wheats, have different physicochemical properties (Dreher 1987). The differences in physicochemical properties of wheat brans could also be influenced by different environmental conditions under which the wheats are grown.

Wheat bran contains more vitamins than any other part of a wheat kernel (Table 2.1). Wheat bran is also rich in dietary fiber especially the insoluble type (Zook et al 1970; Kent 1974). Nutritional studies indicate that insoluble dietary fiber can act as a bulking agent, increasing intestinal motility and wet fecal mass (Cho and Clark 2001). Decreased intestinal residence time and increased amount of stool are associated with reduced incidence and number of colonic tumors (NRC 1989). Wheat bran is known for its effectiveness in the treatment of constipation and other maladies of the colon (Burkitt 1977). The ability of wheat bran to increase stool volume and improve laxation more than any other fiber supplement (Table 2.2) is attributed to its high proportion of insoluble fiber (Mongeau et al 1990; Ranhotra et al 1991; Hosig et al 1996).

Although consumption of wheat bran may provide a number of health benefits, its use and application in food processing remain a challenge as it has poor functionality and/or detrimental effects on end use quality of food products. For example, the use of wheat bran in breadmaking resulted in reduced loaf volume and increased crumb darkness (Lai et al 1989). Grinding of fiber and extrusion processing with high temperature, high shear, and pressure to modify the

physicochemical properties of high fiber ingredients, such as wheat bran, could be an alternative technique for improving their functionality.

Table 2.1. Vitamin Contents (µg/g dry basis) of Hard Red Spring Wheat and its Milling Fractions

Vitamin	Grain	Flour	Shorts	Bran
Thiamine	9.9	0.7	10.1	13.2
Riboflavin	3.1	1.5	1.8	5.5
Niacin	48.3	9.5	23.5	171.4
Biotin	0.056	0.013	0.055	0.162
Folacin	0.56	0.09	0.59	1.59
Pantothenic acid	9.1	2.5	7.0	31.7
Vitamin B6	4.7	0.48	5.3	13.0

Source: Zook et al (1970).

Table 1.2. Effect of Fiber Supplements on Fecal Bulk

Fiber Supplement	Increase in Fecal Weight (%)
Oat bran	15
Pectin	16-35
Guar Gum	20
Apple	40
Carrot	59
Cabbage	67
Cellulose	75
Wheat bran	80-127

Source: Cummings (1997)

2.2. DIETARY FIBER

Dietary fiber is an important food component that has been found to provide positive health benefits. It is able to improve physiological digestive functions, for example, increasing fecal output, and normalizing blood glucose and lipid levels. The nutritional benefits have prompted consumers to develop an interest in whole grains because they contain substantial amounts of dietary fiber and other associated nutrients (Nelson 2001). The most common dietary fiber constituents are depicted in Table 2.3.

According to the American Association of Cereal Chemists International (AACCI), "dietary fiber is defined as the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation" (AACCI 2001). This AACCI definition is a modification of the definition developed by scientists in the mid-1970s who defined dietary fiber as plant cell wall material that is resistant to hydrolysis by the enzymes of the human gastrointestinal tract (Burkitt et al 1972; Trowell 1974; and Painter 1975).

Table 2.2. Constituents of Dietary Fiber

Non-Starch Polysaccharides and Resistant Oligosaccharides
Cellulose
Hemicellulose
Arabinoxylans
Arabinogalactans
Polyfructoses
Inulin
Oligofructans
Galactooligosaccharides
Gums
Mucilages
Pectins
Analogous carbohydrates
Indigestible dextrins
Resistant maltodextrins
Resistant potato dextrins
Synthesized carbohydrate compounds
Polydextrose
Methyl cellulose
Hydroxypropylmethyl cellulose
Resistant starches
Lignin
Substances Associated with the Non-Starch Polysaccharide and Lignin Complexes in Plants
Waxes
Phytates
Cutin
Saponins
Suberin
Tannins

Source: AACCI (2001)

2.2.1. Insoluble Dietary Fiber

Like soluble dietary fiber, insoluble dietary fiber is a type of fiber that cannot be hydrolyzed by the enzymes of the alimentary canal. However, insoluble dietary fiber can be partially fermented by colonic bacteria. Insoluble dietary fiber is commonly used to treat constipation (Nelson 2001). The major components of insoluble dietary fiber are cellulose, certain hemicelluloses, and lignin (Esposito et al 2005).

2.2.1.1. Cellulose

Cellulose is the major structural component of higher plant cell walls such as wheat, barley, rye, oat straw, corn stalks and sugar cane and therefore the most common organic material on earth (Klemm et al 2005; Nelson 2001; Izydorczyk et al 2005). It is a high molecular weight, linear, insoluble homopolymer of D-glucopyranosyl residues linked together in β-1,4 glycosidic linkages (French et al 1993). Its degree of polymerization ranges from 300 to 15,000 units (Donde and Miller 1985). The size of cellulose depends on the source of cellulose and the method of isolation. The β-1,4 linkage between glucose units enables the cellulose to form strong intermolecular and intramolecular hydrogen bonding that result in strong and inflexible crystalline microfibrils of up to 25 nm in diameter (McDougall et al 1996). The unique arrangement of glucose units in cellulose makes it insoluble in hot and cold water, dilute acid, and dilute alkali (Cho et al 1997). Because the human digestive system lacks specific enzymes to hydrolyze β-1,4 linkages, this makes cellulose a truly unavailable carbohydrate (Dreher 1987). Cellulose can be physically and chemically modified to improve its functionality in food systems (Izydorczyk et al 2005). Modified celluloses include microcrystalline cellulose, carboxymethylcellulose, and methylcellulose. Microcrystalline cellulose is usually prepared by treating cellulose with

hydrochloric acid to partially dissolve and remove its less organized amorphous regions. The crystalline aggregates created can be converted to powder form after drying. Carboxymethylcellulose which is usually used as a thickener or stabilizer is prepared by reacting cellulose, alkali, and monochloroacetic acid. Its solubility is enhanced by presence of sodium ions. On the other hand, methylcellulose is a non-ionic ether with thickening and film properties. Methylcellulose is usually prepared by reacting cellulose with methyl chloride (Izydorczyk et al 2005).

2.2.1.2. Hemicelluloses

Scientists unfamiliar with fiber terminology may wrongly assume that hemicelluloses are similar to cellulose (Cho et al 1997; McDougall et al 1996). The designation of *hemi*, which means *half*, was used when early researchers in the area of plant polysaccharides believed that hemicelluloses were on the way to becoming cellulose. Later, hemicelluloses were found to be polysaccharides unrelated to cellulose (Whistler and BeMiller 1997). Both cellulose and hemicellulose are insoluble in hot and cold water, and dilute acid. However, hemicelluloses are distinguished from cellulose because of their solubility in dilute solutions of bases (Dreher 1987).

Hemicelluloses are not structurally related to cellulose. Unlike cellulose that consists of polymers of only glucose units, hemicelluloses are heterosaccharides that have a much lower degree of polymerization ranging from 50-200 residues (Cummings 1976; Theander and Aman 1979). Hemicelluloses contain a variety of monosaccharides in the backbone and side chains. Xylose, mannose, and galactose usually make up the backbone in the beta-1,4 glycosidic configuration. Arabinose, galactose and uronic acids predominate the side chains (Nelson 2001). The number of sugars in the side chains varies such that some hemicelluloses are relatively linear

while others are highly branched. Highly branched hemicelluloses that contain acidic sugars, for example glucuronic acid, in their side chains are actually water soluble.

The major groups of hemicelluloses in cereals include arabinoxylans, glucuronoarabinoxylans, and β-glucans. Arabinoxylans are composed of pentoses, arabinose and xylose (Nelson 2001).

2.2.1.3. Lignin

Although lignin is a component of insoluble dietary fiber, it is not a polysaccharide (Nelson 2001). Lignin is a high molecular weight aromatic polymer composed of polyphenylpropane units which are formed by condensation of the three primary alcohols during plant growth. These alcohols are coniferyl, sinapyl and p-coumaryl (Dreher 1987). Lignin infiltrates the cellulose of cell walls resulting in a hard, rigid matrix of a tremendous strength. Lignin is closely associated with cellulose and hemicellulose (Theander and Aman 1979) and is able to form covalent bonds with them (Southgate 1995). Lignin is highly insoluble in water and is responsible for structural adhesion of the plant cell wall components because it excludes water when it forms networks by cross-linking with other saccharide-type molecules in plants. Lignin is very resistant to chemical, enzymatic and bacterial breakdown. Thus, lignin resists enzymatic breakdown in the small intestine and bacterial breakdown in the large intestine and can be recovered almost completely intact in the feces (Cho et al 1997).

2.2.2. Soluble Dietary Fiber

Soluble dietary fiber is different from insoluble dietary fiber because of its solubility in aqueous solutions of the digestive system. It is important to note that solubility of fibers is quite

different from solubility of sugars such as glucose. Fiber is never completely dissolved in aqueous solution. Instead, it behaves as a colloid (Nelson 2001). Soluble fiber mainly consists of pectins, certain hemicelluloses, gums, and β-glucans (Esposito et al 2005).

2.2.2.1. Arabinoxylans

In wheat grain, arabinoxylans are believed to be the major components of cell walls (Saulinier et al 2007). Arabinoxylans are comprised of a linear backbone of β-1,4-linked D-xylopyranosyl units. α-L-arabinofuranosyl and α-D-glucuronic acids are attached to the backbone on oxygen 2 (O-2) or oxygen 3 (O-3) as the major side chains (Dervilly et al 2000; Saulinier et al 2007). Ferulic and p-coumaric acids are esterified to the α-L-arabinofuranosyl units through oxygen O-5 (Smith and Hartley 1983; Saulinier et al 2007).

The amount of arabinoxylans in wheat flour ranges from 0.3 to 0.7% (w/w). Although they are not major constituents in wheat flour, arabinoxylans are known to have significant influence on dough rheology as well as on baking properties (Biliaderis et al 1995). Unlike in cell walls, the arabinoxylans in wheat flour (endosperm) are composed of only arabinose and xylose. These sugars contain five carbons and are therefore referred to as pentosans. There are two types of arabinoxylans: water-extractable (WE-AX) and water- unextractable (WU-AX). The amounts of WE-AX and WU-AX in wheat flour are 0.5% and 1.7% (w/w), respectively (Dervilly-Pinel et al 2001; Ordaz-Ortiz and Saulinier 2005; Saulinier et al 1995).

The structure of arabinoxylans in the residual starch (aleurone layer) adhered to wheat bran after roller milling is different from that in the endosperm. Arabinoxylans in the aleurone layer are not water-extractable and have a lower arabinose to xylose ratio than the arabinoxylans

in the endosperm (Antoine et al 2003). Also, the esterification of arabinoxylans is more pronounced in the aleurone layer than in the endosperm (Parker et al 2005).

The functionality of arabinoxylans heavily depends on their physicochemical properties such as viscosity and water solubility (Saulinier et al 2007). It is important to understand that the structure and conformation of polymers, such as chain length and composition, including side chains, are the major factors influencing the physicochemical properties of arabinoxylans (Saulinier et al 2007).

2.2.2.2. β-glucans

Beta-glucans have gained popularity because research has shown that consumption of β-glucan reduces serum cholesterol (Tapola and Sarkkinen 2007). In addition, β-glucan is of interest because of its ability to attenuate blood glucose levels (Wood et al 1989). Beta-glucans are non-branched polymers of glucopyranosyl units with a mixture of β-1,3 and β-1,4 linkages (Wood 1989). Grains, especially oats and barley, contain high amounts of beta-glucans (Wood et al 1989). β-glucan content (dry weight basis) in oats and wheat range from 2.5% to 6.6% (w/w) and 0.5% to 1.5% (w/w), respectively (Table 2.4). However, these values depend on environmental factors and genetic make-up of oat cultivars (Nelson 2001). The amounts of β-glucan in barley and rye are also listed in Table 2.4. β-glucans are often referred to as food gums or mucilages since they hydrate easily, forming viscous solutions (Dziezak 1991). Thus, β-glucans are grouped as hydrocolloids since they have the ability to form a gel after dispersion in water. Other types of hydrocolloids include alginates, carrageenan, gums Arabic, karaya, tragacanth and xanthan gum (Dziezak 1991). Beta-glucans form long cylindrical molecules containing up to 250,000 glucose units. The presence of β-1,3 and β-1,4 linkages prevents the compact folding of β-glucans, hence making them soluble in water (Chawla and Patil 2010).

Table 2.3. β-Glucan Content (dry weight basis) of Selected Cereal Grains

Grain	β-glucan (% w/w)
Barley	2.0 - 9.0
Oats	2.5 - 6.6
Rye	1.9 - 2.9
Wheat	0.5 - 1.5

Source: Cho et al (1997)

2.2.2.3. Pectin

Pectin is a component of higher plant cell walls where it is used for overall growth of plant cells. It is mostly found in the middle lamella and is used for ion transport and water retention. It is considered the largest source of soluble fiber among plant food materials and is used to stabilize food systems due to its high viscosity properties (Endress and Mattes 2009).

The backbone of pectin is composed of α-1,4-linked galacturonic acid residues interrupted by single α-1,2-linked rhamnose residues (Voragen et al 1995; BeMiller 1986). The side chains of pectin consist of galactose, glucose, rhamnose, and arabinose (Cho et al 1997; McDougall et al 1996; Dreher 1987). Solubility of pectins depends on the side chain composition (Dreher 1987). Pectins are among the primary cell wall components where they are linked to cellulose, hemicellulose, and lignin (Lopes da Silva and Rao 2006) and are a key textural determinant in fruits and vegetables (Voragen et al 1995; Wang et al 2002). Pectin is the major soluble fiber in fruits and vegetables. However, in wheat, it is present in very small amounts.

2.2.3. Non-Digestible Oligosaccharides

Foods that have the ability to reduce the risk of diseases and provide better health have become popular as consumers continue to become health conscious. In this respect, significant attention has been paid to a group of carbohydrates commonly known as the nondigestible oligosaccharides (NDOs) (Mussatto and Mancilla 2007). NDOs are not digestible by humans because the human digestive system lacks the enzymes required to hydrolyze the β-links formed among the units of some monosaccharides (Urgell and Orleans 2001). These oligosaccharides are then fermented by beneficial bacteria in the colon where they stimulate the growth and activity of beneficial bacteria and concomitantly repress harmful bacteria (Mussatto and Mancilla 2007). The

principal metabolites of fermentation are lactate and short-chain fatty acids such as acetate, propionate and butyrate. These short-chain fatty acids are believed to: 1) increase water and sodium absorption from digesta in the colon, 2) facilitate mucosal cell proliferation, 3) provide energy for colonic microorganisms, and 4) acidify lumenal environment. The amounts of short-chain fatty acids produced in the colon depend on the type of NDO substrates and types of intestinal flora (Sako et al 1999). The rate at which oligosaccharides are fermented also depends on their degree of polymerization (Manning and Gibbson 2004).

2.2.4. Resistant Starch

Resistant starch is a type of starch that cannot be hydrolyzed by the enzymes in the digestive system, especially the small intestine of humans. Thus, this type of starch cannot be absorbed by humans. However, resistant starch can be fermented in the large intestine. Resistant starch is considered a form of dietary fiber due to the fact that it is not attacked by the enzymes in the small intestine and undergoes fermentation in the large intestine (Grooper et al 2009; Nelson 2001).

Resistant starch is categorized as RS1, RS2, RS3, and RS4. RS1 is a type of starch that is trapped in the cell walls of plants and therefore cannot be attacked by hydrolyzing enzymes. These starches can only be hydrolyzed by breaking the system in which they are trapped. Sources of RS1 include partially milled grains, seeds and legumes (Gropper et al 2009; Yue and Waring 1998). RS2 encompasses starches that are intact granules and not gelatinized. These starches can be hydrolyzed by enzymes after the process of gelatinization. Sources of RS2 starches include raw potatoes and unripe green bananas. RS3 is a type of starch that is formed after cooking starches under high moisture conditions. The ideal factor for the formation of RS3 is

gelatinization and retrogradation. Enzymes cannot hydrolyze this type of starch because of the network formed through crystalline junction zones as a result of retrogradation. Extrusion cooking under high moisture is one of the popular cooking methods used to increase RS3 in foods. Sources of RS3 include ready-to-eat cereals, and cooked and cooled potatoes and breads. RS4 is a type of resistant starch formed by chemical modification of starches, for example, formation of ester bonds. RS4 cannot easily be broken down by digestive enzymes because the enzyme-attacking sites are taken up by crosslinking chemicals (Grooper et al 2009; Nelson 2001).

2.2.5. History of Dietary Fiber

For many years, dietary fiber has been known to provide health benefits. For example, consuming foods high in indigestible carbohydrates improved bowel habits and increased fecal volume. The term dietary fiber was later used to describe non-digestible plant components such as cellulose, hemicelluloses and lignin (Hipsley 1953).

In the mid-1970s, there was sufficient data to suggest that consumption of foods high in fiber could provide health benefits. Studies conducted in Africa showed that Africans who consumed foods high in fiber had frequent and bulky stools and had fewer of the illnesses that were common in western culture. It was eventually hypothesized that the indigestible plant constituents were able to provide health benefits (Burkitt et al 1972; Trowell 1974; and Painter 1975).

In 1976, dietary fiber was redefined to include all indigestible polysaccharides, including gums, modified celluloses, mucilages, oligosaccharides, and pectins. This new definition came after new information about the chemical structures of dietary fibers was obtained. This definition became widely accepted (AACCI 2001).

Understanding the chemical composition and health benefits associated with dietary fiber prompted several researchers to develop methods to determine dietary fiber content in foods (Southgate 1969; Baker 1979; Asp and Johanson 1981). The major purpose of the dietary fiber content determination method was to remove all digestible components of foods from the non-digestible components using specific enzymes.

In the late 1970s, scientists sought consensus on a definition of dietary fiber that matched with its health benefits (Prosky and Harland 1979). The consensus was achieved in 1981 during the Spring Workshop of the Association of Official Analytical Chemists in Ottawa, Canada (Prosky 1981).

2.2.5. Health Benefits of Dietary Fiber

Since the middle of the 1970s when Burkitt and Trowell first developed a dietary fiber hypothesis, the role of dietary fiber has attracted significant attention in the area of health and nutrition (Hamid and Luan 2000; Marlett et al 2002). The hypothesis put forward by Burkitt and Trowell stated that consumption of foods low in dietary fiber was the major cause of chronic diseases such as heart disease, diabetes, diverticulitis, and certain types of cancers that were common in western societies. Medical data indicate that consuming diets high in dietary fiber is associated with health benefits, especially in Western populations (McCleary 2007). Consumption of foods high in insoluble dietary fiber improves bowel function and may reduce the symptoms of chronic constipation, diverticular disease and hemorrhoids. Several investigators have reported that the prevalence of Western diseases, including high cholesterol and cardiovascular disease (Jones 2008), colon cancer (Drecker et al 2002), diabetes, obesity, and constipation, can be reduced by consuming fiber-enriched foods (Kantor et al 2001). Although several studies have

shown that consumption of foods high in dietary fiber is inversely proportional to the occurrence of chronic diseases, it is not clear if health benefits are due to dietary fiber per se or other chemical components, for example, antioxidants that are associated with dietary fiber (Jones 2008).

Despite the above mentioned health benefits of dietary fiber, its consumption remains low, especially in the United States. Recommended dietary fiber intake in the United States ranges between 20 and 35 grams per day, but the average intake is between 14 and 15 grams per day (Cho 2009). In other developed countries, such as European countries, the average consumption of fiber is also below recommended intakes (Murakami et al 2007).

Based on published data implicating the role of dietary fiber in reducing the risks of degenerative diseases, the Food and Drug Administration (FDA) of the United States approved a number of claims that certain types of fiber may reduce the risk of cancer and coronary heart disease (FDA 1993; FDA 1998).

2.3. PHYSICOCHEMICAL PROPERTIES OF HIGH FIBER INGREDIENTS

2.3.1. Solubility

Solubility is considered a major physicochemical property of dietary fiber (Oakenfull 2001). In fiber terminology, solubility does not mean that the fiber in question is completely dissolved in water as in a fashion similar to glucose (Nelson 2001). Solubility rather means that the fiber components are easily hydrated in water to form a colloidal suspension. Thus, the solubility of high fiber ingredients refers to the state of the ingredient in water and not the solubility of an ingredient in water.

Molecular structure is the major factor that affects solubility of polysaccharides (Morris and Norton 1983). Branching in the backbone of a polymer can increase the polymer's solubility (Nelson 2001). Branching disrupts the intermolecular forces, thereby preventing the formation of ordered crystalline structure. Examples of branched polysaccharides that are soluble include gum acacia and arabinogalactans (Nelson 2001). On the other hand, cellulose, which is characterized by linear and ordered crystalline structure stabilized by hydrogen bonds, is water-insoluble (Morris 1989).

Polysaccharides with ionizing groups, for example pectins and carrageenans, are easily dissolved in water. Electrostatic repulsion inhibits the high fiber components from close packing, thus preventing the formation of ordered structures that tend to be insoluble in water (Patil 2008). The solubility of a polysaccharide is also determined by the nature of linkages between its monomers (Morris and Norton 1983). For example, the mixed β-1,3 and β-1,4 linkages of β-glucans prevent the formation of linear crystalline structure, thereby enhancing hydration (solubility). Cellulose, which consists of D-glucose entirely linked by β-1,4 glycosidic bonds, is insoluble in water.

2.3.2. Water Binding Capacity

The term water binding capacity simply refers to the way in which polysaccharides interact with water (Patil 2008). Other terminologies include water uptake, hydration, adsorption, and absorption. The most commonly used terminologies are water binding capacity and water holding capacity. Based on the definitions given by Rey and Rabuza (1981), water holding capacity is the amount of water the system retains within its structures without subjection to any given addition of pressure or stress. On the other hand, water binding capacity is the amount of water a system retains after it has been subjected to stress, for example centrifugation.

Several factors influence the water holding capacity of high fiber ingredients, for example, the source of the raw material, processing conditions, and the way it is measured (Patil 2008). The ability of fiber to increase stool volume and get fermented in the large intestine depends on water absorption properties.

2.3.3. Bile Acid Binding Capacity

Bile acids are made in the liver from cholesterol. They are conjugated with glycine or taurine and are stored in the gallbladder. Upon eating a meal, the contents of the gallbladder are secreted into the intestine where the bile acids serve the purpose of emulsifying fats. About 90% of the excreted bile acids are reabsorbed by active transport in what is referred to as active enterohepatic circulation (Hofmann 1977). Binding of bile acids and increasing their fecal excretion has been hypothesized as a possible mechanism for lowering cholesterol by dietary fiber (Lund et al 1989; Anderson and Siesel 1990). By binding bile acids, dietary fiber prevents reabsorption and stimulates plasma and liver cholesterol conversion to additional bile acids (Kritchevsky and Story 1974). Soluble fiber is known to be effective in reducing blood

cholesterol (FDA 1998; Seiz 2006). Previous investigators (Ralet 1990; Esposito et al 2005; Gajula et al 2008) were able to increase the proportion of soluble fiber in wheat bran by extrusion cooking. However, it is not known whether the soluble fiber induced by extrusion cooking has the same cholesterol-lowering effects as naturally soluble fiber.

2.3.4. Molecular Weight Distribution

Molecular weight is one of the important characteristics that affect physical properties, such as solubility and viscosity, of polysaccharides (Wang and Cui 2005). Polysaccharides are made of monosaccharides of different sizes and structures and thus they are polydisperse and polymolecular (Wang and Cui 2005; Saulnier et al 2007). In addition to different sizes and structures, the numbers of monosaccharides that make up a polysaccharide vary, resulting in a distribution of molecular weight for that particular type of polysaccharide (Wang and Cui 2005). The apparent molecular weight of a polysaccharide depends on the pathway and environment of synthesis, extraction conditions used to isolate the polysaccharide, and the method of molecular weight measurement (Wang and Cui 2005). To better describe the molecular weight of a polysaccharide, information on the average molecular weight is needed. The average molecular weight is described by molecular weight averages, such as number average molecular weight (Mn), weight average molecular weight (Mw), z-average molecular weight (Mz), and viscosity average molecular weight (Mv). Mn is related to the chain length of a polysaccharide. It can be physically measured by counting the number of molecules that make up a polysaccharide, for example, by using membrane osmometry. Mn is commonly known to influence the thermodynamic properties of polysaccharides. Mw measures the size of the polymer but by emphasizing heavier molecules than Mn. The most common technique for measuring Mw is light

scattering and sedimentation equilibrium. Mz is a third power average, which is commonly determined by sedimentation techniques. Mz, which is determined by sedimentation techniques, refers to the melt elasticity of polysaccharides. Mv, which is the viscosity-average molecular weight, is a measure of intrinsic viscosity of a polysaccharide and is related to molecular weight (Wang and Cui 2005).

The effect of processing, such as extrusion cooking, may have a significant effect on the molecular weight distribution. The mechanical shear and high temperature of extrusion can cause degradation of a polysaccharide, thus affecting its molecular weight distribution.

2.3.5. Thermal Analysis

Thermal analysis is a family of methods that is used to monitor thermal events of a sample as a function of temperature and time. There are several thermal analysis techniques available: differential thermal analysis (DTA), differential scanning calorimetry (DSC), dynamic mechanical analysis (DMA), thermogravimetric analysis (TGA), and thermomechanical analysis (TMA) (Schenz 2003). Of these techniques, DSC is the most widely used technique to characterize polysaccharides. Information provided by DSC measurements includes the temperatures for glass transition (Tg) and melting (Tm). DSC also measures changes in enthalpy (ΔH) during melting. Thermal analysis techniques can provide qualitative and quantitative data about any physical and chemical changes in a polysaccharide that involve endothermic and exothermic processes.

There are a number of physical events that occur during heating and cooling of a polysaccharide. For example, endothermic transition occurs when heat flows into a sample

causing disruption of ordered structures. Exothermic transition occurs when heat is released from a sample system during the formation of ordered structures.

2.4. EFFECT OF PROCESSING ON DIETARY FIBER

Processing can alter sensory attributes, nutritional profile, safety, and stability of the fiber containing product. Processing results in molecular, structural and functional changes which in turn alter the physicochemical properties of fiber ingredients. Understanding how processing affects physicochemical properties would help to understand how fibers induce physiological effects (Guillon and Champ 2000). This can in turn provide a good link between food technology and nutrition. Several scientists are interested in understanding how processing affects nutritional properties of food products (Guillon and Champ 2000).

Mechanical processing, such as grinding, can cause significant changes in the physicochemical properties of dietary fiber ingredients, for example, the hydration properties of fiber (Zhang and Moore 1997). Wood et al (1989) reported that fibers in oats are susceptible to degradation which also results in decreased viscosity.

Extrusion processing, which is commonly used in manufacturing breakfast cereals, can also modify the properties of dietary fiber (Nyman et al 1994). Decrease of soluble dietary fiber with increase in soluble dietary fiber has been reported (McDougall et al 1996). Extrusion processing has the ability to degrade dietary fiber polysaccharides which results in lower molecular weight and lower viscosity (Snavberg 1997). However, these changes depend on the extrusion variables such as moisture content, screw configuration, temperature and screw speed (Camire and Flint 1991; Ralet et al 1991).

2.4.1. Particle Size Distribution

Particle size distribution is one of the major factors that determines both fiber's functionality and fiber's role in the digestive tract (transit time, fermentation, fecal excretion). The

shape, and consequently the size of the fibers, depends on the degree of processing and this shape may also vary during transit in the intestine tract as a result of digestion processes (Rosell et al 2009). Heller et al (1980) found out the mean transit time of coarse wheat bran was significantly shorter compared to that of finely ground wheat bran. Chemical composition of fibers is also thought to affect their physiological functions such as fecal bulking: the more pentose sugars present in the dietary fiber polymers, the greater the increase in stool weight (Cummings et al 1978).

It is known that coarse wheat bran fiber has a higher water holding capacity, which is directly related to its physiological functions such as increasing stool bulk and decreasing mean transit time. However, the notion that water holding capacity will explain how various fibers will behave in the digestive tract needs to be critically analyzed. It is possible that fibers may break down in the digestive tract.

Reduction of particle size can have varying effects on the physical structure of different fibers. Grinding can reduce the particle diameter and collapse the fiber matrix of wheat bran (Cadden 1987). Particle size distribution of wheat bran affects physical and sensory properties of bread: coarse wheat bran resulted in darker crust, dull crumb and decreased loaf volume (Zhang and Moore 1999). Panelists rated bread containing coarse bran as less acceptable.

2.4.2. Extrusion Cooking

Extrusion cooking is a thermo-mechanical process that applies high temperature, high pressure, and shear to raw food ingredients (Heldman and Hartel 1997). Extruders have gained popularity in recent years due to their versatility, high throughput, low-cost, product quality, energy savings, and addressing of environmental concerns (Riaz 2001). Extrusion cooking is also

a high-temperature short-time technique. It is a short-time processing technique since the materials stay in the extruder barrel for only a very short time relative to traditional cooking methods.

The physicochemical properties of extruded products mainly depend on how extrusion cooking variables are manipulated (Huber 2000; Ryu and Ng 2001). In extrusion, independent variables refer to process parameters that the extruder operator can control. The major independent variables are screw speed and screw configuration, amount of water injected into the extruder barrel, feed rate, die size and die configuration. On the other hand, dependent variables refer to the parameters that are influenced by independent variables. Some of the major dependent variables include the mean residence time, pressure inside the extruder barrel, and the mechanical energy input to the extruder (Huber 2000).

Screw speed and configuration are the major operating (independent) variables of the extrusion cooking process. Screw speed can be used to regulate the power input to the material in the extruder (Altimore and Ghossi 1986). In rotational systems, power is related to the torque and angular velocity. Thus, increasing screw speed increases energy to the system and vice-versa. Changing screw speed changes the degree of fill inside the barrel, the mean residence time, and the shear stress on the material being extruded (Altimore and Ghossi 1986). The high shear in the extruder causes mechanical modification of the extruded product (Riaz 2001).

Moisture is very critical during the extrusion cooking process (Huber 2000). The amount of water injected into the extruder barrel influences the material in the extruder (Altimore and Ghossi 1986; Heldman and Hartel 1997). Moisture in the raw materials is required during extrusion cooking for starch gelatinization and protein denaturation. Increasing the moisture decreases mechanical energy of the extruder (Altimore and Ghossi 1986; Huber 2000).

Feed rate, the amount of material that is fed into the extruder per unit time, has a significant effect on the quality of the extruded product. Feed rate has a dramatic influence on the mean residence time (Altimore and Ghossi 1986). The higher the feed rate, the shorter and more uniform the residence time distribution. Using a hopper that feeds a constant uniform amount of the dry material into the extruder allows production of a consistent and uniform extruded product (Heldman and Hartel 1997). Inconsistent feeding may lead to inconsistent quality in the final product.

Significant changes can take place in the polysaccharides plant cell walls during food processing. These changes can have an impact on the physicochemical and nutritional properties of the dietary fiber such as redistribution of soluble and insoluble fibers in total fiber with a proportionate increase in soluble fiber. This could improve the hypocholesterolemic properties of fiber. Extrusion cooking significantly increased the proportion of soluble fiber in extruded wheat bran (Aoe et al 1989; Ralet et al 1990; Wang and Klopfenstein 1993; Gualberto et al 1997).

Thermo-mechanical effects during extrusion processing can have a profound effect on the composition of dietary fiber. Applying high temperature and shear are believed to weaken and break the glycosidic linkages holding polysaccharide monomers together. As a result, the structure of the fiber may be altered and allow insoluble fiber to become soluble.

Contradicting results also have been published. Artz et al (1990) reported that extrusion cooking did not result in significant changes in the amount of soluble and insoluble fibers in wheat bran. However, it is important to note that the source of fiber, the way it is prepared before processing, and the processing conditions themselves are all crucial when studying the effect of extrusion cooking and other processing techniques on the physicochemical properties of dietary fiber (Guillon and Champ 2000).

2.5. WHEAT FLOUR

Among food grains, wheat is one of the most extensively produced and consumed cereals. Three common species of wheat are *Triticum aestivum*, *T. compactum*, and *T. durum*. There are five classes of *T. aestivum* (hard red winter, hard red spring, soft red winter, hard white, and soft white). *T. compactum* includes the club wheat while *T. durum* forms the class of durum wheat (Artwell 2001). Wheat, mainly as flour, is utilized for the preparation of a wide range of products such as bread (leavened and unleavened), cakes, biscuits, cookies, crackers, and others. Wheat is a good source of vitamins B1 (thiamine), B2 (riboflavin), niacin, B6 (pyridoxine), pantothenic acid, tocopherol, as well as iron and zinc (Hoseney 1994).

Based on 14% moisture content, wheat flour is mainly composed of starch (63-72%, w/w) and protein (7-15%, w/w). The amounts of starch and protein also depend on wheat cultivar and growth environments. In addition to protein and starch, wheat flour contains other polysaccharides such as hemicellulose, pentosans, arabinose, xylose, and arabinoxylan. Wheat flour also contains some lipids and ash.

Starch is the largest molecule in the world after cellulose. Functionality of starch depends on its botanical source. Starch, the major polymer in wheat flour, is composed of two groups of molecules: amyloses and amylopectins (Tester et al 2004). Amylose is believed to be a linear polymer of glucose units joined together at carbons 1 and 4 (α-1, 4 linkage). Usually in a single amylose molecule there are 1500-6000 glucose units (Atwell 2001) with a molecular weight of approximately 1×10^5 to 1×10^6 (Tester et al 2004). This number depends on the wheat species (Hoseney 1994). Starch is insoluble in cold water but dissolves in warm water (about 60^oC). Amylose is responsible for starch retrogradation. Amylose molecules tend to associate with

themselves after gelatinization and precipitate out of the solution. Common wheat starch contains approximately 25% amylose (Atwell 2001).

There are two ways in which amylopectin is distinguished from amylose. First, it is a much larger molecule compared to amylose; a single amylopectin molecule contains 300,000–3,000,000 glucose units. Second, glucose units in amylopectin are linked together by α-1, 4 and α-1, 6 glycosidic linkages. Common wheat starch contains approximately 75% amylopectin (Atwell 2001). This amount depends on the source of wheat variety.

Wheat proteins are classified based on their solubility, and approximately 80% of wheat flour proteins are gluten proteins (Atwell 2001). In the early 1900s, Osborne classified wheat proteins as albumins, globulins, prolamins, and glutenins. Albumins are soluble in water and make up 15% of flour protein. Albumins are comparable to egg white protein. The functionality of albumins is not easily affected by salt concentrations (Hoseney 1994). Globulins are the minor proteins in wheat. They make up only 3% of the total flour protein. They are insoluble in water, but solubility can be achieved at low salt concentrations. However, solubility of globulins is decreased by higher salt concentrations. Globulins are the only wheat proteins that exhibit the salting in and salting out effects. Prolamins are cereal proteins that are soluble in 70% ethanol whereas some glutenins are soluble in dilute acids and bases.

2.6. BAKED PRODUCTS

The most common baked products available on the world market include breads, cakes, pastries, cookies, crackers, muffins, and several other products. It is important to note that all the products mentioned above have one ingredient in common: it is wheat flour. Most of the baked products have wheat in the form of flour as a major ingredient. Wheat is one of the most extensively produced and consumed cereals in the world (Shenoy and Prakash 2002). Couvain and Young (2006) define baked products as "foods manufactured from recipes largely based on or containing significant quantities of wheat or other cereal flours which are blended with other ingredients, are formed into distinctive shapes and undergo a heat processing step which involves the removal of moisture in the oven."

Wheat is subdivided into hard and soft wheat. Hard wheats are high in gluten proteins and therefore preferred for bread making. On the other hand, soft wheats are low in gluten proteins and are thus good for non-bread products such as cakes, cookies, crackers, muffins, and other soft wheat-based products.

Wheat bran is regarded as the major source of dietary fiber in baked products. However, addition of wheat bran during the breadmaking process causes major changes in dough properties. These changes can lead to detrimental effects such as reduced bread volume and increased crumb hardness (Rosell and Foegeding 2007). Incorporation of wheat bran during cookie making results in poor quality cookies including reduced spread during baking (Uysal et al 2007; Gujral et al 2003; Tangkanakul et al 1995).

The basic raw material for most cereal-based baked products is wheat in the form of flour. Wheat flour obtained after a dry milling process is required as a starting point of most bakery processes. With baked products, wheat flour has to have other raw materials added to it to form

doughs/batters before baking. The other ingredients combined with wheat flour in baked products recipes impart considerable changes to the functionality of wheat flour. For example, sugar restricts availability of water which reduces gluten-forming potential of wheat proteins and modifies starch gelatinization as well. Sugar also acts as a substrate for yeast to produce carbon dioxide that is responsible for the rising of the dough during proofing to produce a leavened produt (Cauvain 2001). In addition, sugar provides sweetness to a variety of baked products including bread, cake, muffins, and biscuits.

In general, hard wheat flour is widely used for making leavened bread whereas soft wheat flour is suitable for non-leavened baked products such as cookies and cakes. The quality of end-use baked products depend on the physicochemical properties of flour used (Cauvain 2001). Addition of fiber to bread or cookie formulation affects the physicochemical properties and sensory attributes of baked products (Nelson 2001).

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CHAPTER 3

PHYSICOCHEMICAL PROPERTIES OF WASHED WHEAT BRAN

3.1. ABSTRACT

Wheat bran, a by-product of roller milling during the milling process of wheat, contains substantial amounts of residual starch that may interfere with the analysis of bran's physicochemical properties. The specific objectives of this study were to develop a method that washes away most of the starch adherent to milled wheat bran and to investigate the effects washing has on the physicochemical properties (such as water binding capacity) and composition (including insoluble dietary fiber, soluble dietary fiber, total dietary fiber) of washed and non-washed wheat bran. Soft white wheat bran was washed with distilled water at room temperature and mixed with a modified Servodyne mixer to wash residual starch away from bran. The bran-starch slurry was transferred into a SoyCow presser lined with a filter cloth and rinsed to remove as much starch as possible. The washed and non-washed bran samples were dried overnight at 60^{o}C and ground to pass through 1000 or 425 μm screens. Washing significantly reduced starch adherent to wheat bran by 76% (w/w), changed contents of insoluble dietary fiber and soluble dietary fiber from 39 to 69% (w/w) and from 4.93 to 1.68% (w/w), respectively. Water binding capacity was higher for washed bran, and was not affected by bran particle size. The transition onset and peak temperatures of washed wheat bran samples were significantly higher than the counterpart values of non-washed bran samples. On the other hand, transition enthalpies of washed bran samples were lower than those of non-washed bran samples.

3.2. INTRODUCTION

In recent years, dietary fiber has attracted significant attention due to health care officials' and nutritionists' recommendations that consumption of dietary fiber can help to maintain good health (McCleary 2007). Dietary fiber reduces the risk of cardiovascular disease, certain forms of cancer, and constipation (Park et al 2005). Dietary fibers are categorized as insoluble and soluble dietary fibers and each plays a different role in human health. Insoluble dietary fiber is important for proper bowel function (Anderson 1985; Anderson and Eastwood 1986; Cummings et al 1978) and may reduce symptoms of chronic constipation, diverticular disease, and hemorrhoids (Painter and Burkitt 1971; Burkitt 1977; Cummings et al 1979). On the other hand, soluble dietary fiber is associated with reduction in cholesterol levels and attenuation of blood glucose (Kantor et al 2001; Drecker et al 2002; Schaafsma 2004; Jones 2008). The physiological functions of dietary fibers are related to their physicochemical properties such as water binding capacity and distribution of insoluble and soluble dietary fibers (Schneeman 1999).

Wheat bran, a by-product of roller milling during the milling process, is rich in dietary fiber and contains substantial amounts of protein, minerals and vitamins (Ortho and Shellenberger 1971). It is used by the baking industry to increase dietary fiber in baked products especially of bread (Pomeranz et al 1977; Vetter 1988). It is important to know that in wheat bran, the amount of soluble dietary fiber is very low compared to that of insoluble dietary fiber, which implies that the physiological effects of wheat bran, such as proper bowel function, are attributable to the insoluble dietary fiber. Increased stool weight and short intestinal transit times are affected by the hydration properties of insoluble dietary fiber (Hill 1983). The ways in which fiber-containing samples are prepared determine the hydration properties of that fiber, for example, the amount of water a fiber sample can hold in its matrix. This, in turn, can also influence a fiber's physiological

functions within the gastrointestinal tract (Rasper 1982). Wheat bran obtained after roller milling contains significant amounts of residual starch still adherent to it. This residual starch, if not removed, can interfere with the analyses that are used to determine the composition and physicochemical properties of wheat bran. Therefore, the major objectives of the present study were to develop a washing method that would remove as much residual starch as possible and then study the effect of washing and particle size distribution on composition and physicochemical properties of relatively pure wheat bran.

3.3. MATERIALS AND METHODS

3.3.1. Wheat Bran Sample

The non-washed wheat bran sample used in this study was milled from soft white wheat of the 2009 crop and supplied by Star of the West Milling Company (Frankenmuth, MI, USA). The sample was stored at 4^{O}C until the studies were conducted.

3.3.2. Washing of wheat bran

Washing of wheat bran was performed in batches. For each batch, 500 g of non-washed wheat bran was added to 5 L of distilled water at room temperature in a 5 gal white plastic bucket (Paragon Molding Company, Melrose Park, IL, USA) and mixed with an electronic Servodyne mixer (Cole-Palmer Instruments Company, Vernon Hills, IL, USA) at a speed of 150 rpm for 30 min to wash residual starch away from bran. The Servodyne mixer was modified by adding mixing paddles as depicted in Figure 3.1. After mixing, the wheat bran and starch slurry was transferred into a stainless steel SoyCow presser (ProSoya Inc., Ottawa, Canada) with a filter cloth (170 µm) laid inside the presser according to the manufacturer's manual. The tap of the presser was opened to drain the starch slurry. Wheat bran on the filter cloth was rinsed with 5 L distilled water a second time to remove as much residual starch as possible. The washed bran (WB) and non-washed bran (NWB) were spread on 45 cm x 66 cm x 2.54 cm aluminum baking trays (WearEver, Millville, NJ, USA) and dried overnight at 60^{O}C in a Proctor dryer (Proctor & Schwartz, Inc., Philadelphia, USA). Dried WB and NWB samples were ground using a hammer mill (Model D Comminuting Machine, W.J. Fitzpatrick Company, Chicago, Illinois, USA) to pass through a 1270 µm screen (Part Number 1532 0050, Model DAS06, W.J. Fitzpatrick Company, Chicago, Illinois, USA). NWB and WB samples were then sifted through 1000 µm

(US mesh #18) or 425 μm (US mesh #40) sieves (Great Western Manufacturing Company, Leavenworth, Kansas, USA) to obtain NWB1000, NWB425, WB1000 and WB425. The samples were placed in one-gallon ziplock plastic bags and stored at 4^{o}C until required for analysis. Hereafter, the ground samples are referred to as washed or non-washed bran samples.

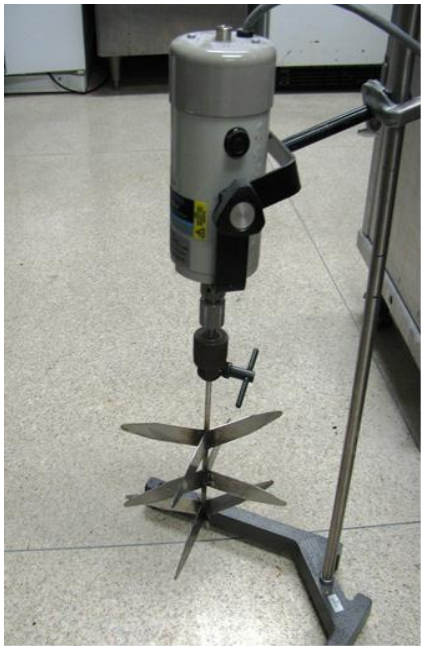

Figure 3.1. Modified servodyne mixer used to wash wheat bran. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

3.3.3. Determination of Total Starch

Total starch content in washed and non-washed bran was determined in triplicate according to the procedure in the Total Starch Assay Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland). Briefly, approximately 100 mg of bran sample were placed in 17 mL glass test tubes followed by addition of 0.2 mL of 80% (v/v) ethanol to aid in dispersing the sample. Three mL of thermostable α-amylase (3,000 U/mL of Ceralpha reagent) diluted (1:30) in sodium acetate buffer (100 mM, pH 5.0) were immediately added to the samples and the tubes were incubated in a boiling water bath (Blue M, Blue Island, IL, USA) for 6 min with vortexing at 2, 4, and 6 min. The tubes were then placed in a water bath (Blue M, Blue Island, IL, USA) at 50^{o}C and 0.1 mL of amyloglucosidase (3300 U/mL of soluble starch) was immediately added to each tube. The samples were incubated for 30 min. After incubation, the contents of the test tubes were transferred to 100 mL volumetric flasks and the volume adjusted with deionized water to 100 mL. The contents of the volumetric flasks were then transferred to 150 mL beakers and 3 mL of the contents were placed in plastic centrifuge tubes and centrifuged at 1800 x g for 10 min at 25^{o}C. For each sample, a 0.1 mL aliquot of the clear supernatant was pipetted to the bottom of a 15 mL test tube, followed by addition of 3 mL of glucose oxidase-peroxidase-aminoantipyrine (GOPOD) reagent. The samples were incubated in the water bath at 50^{o}C for 20 min. A spectrophotometer (Spectronic 5, Spectronic Instruments Inc., Rochester, NY, USA) was used to measure the absorbance for each sample at 510 nm against the reagent blank (0.1 mL of deionized water and 3 mL of GOPOD reagent). Total starch (%) on a dry weight basis was calculated based on formulas outlined in the Total Starch Kit.

3.3.4. Thermal Properties of Non-Washed and Washed Wheat Bran Samples

The thermal properties of non-washed and washed wheat bran samples were studied by a Differential Scanning Calorimetry (DSC Model Q100 V9.9 Build 303, Greifensee, Switzerland). Each sample was weighed into a DSC aluminum pan and 20 µL of distilled water were added using a micro-syringe. The sample was hermetically sealed and the moisture allowed to equilibrate overnight at room temperature. Samples were heated from 20 to 200^{o}C at the heating rate of 10^{o}C/min. A sealed empty pan was used as a reference. Transition onset temperature (T_o), transition peak temperature (T_p), and transition enthalpy (ΔH) were recorded and analyzed using DSC software (Universal V4. 7A, TA Instruments, Newcastle, DE, USA).

3.3.5. Water Binding Capacity of Non-Washed and Washed Bran

Water binding capacity of non-washed and washed wheat bran samples was measured in triplicate according to AACCI Approved Method 56-30 (AACCI 2000) with some modifications. 30 mL of deionized water were added to approximately one gram of bran sample in pre-weighed plastic centrifuge tubes. The tubes were vortex-mixed to ensure that all the bran particles were thoroughly wetted. The samples were allowed to hydrate for 30 min with hand shaking after 10, 20, and 30 min. The tubes were centrifuged (Model J2-21M, Beckman Instruments Inc., Fullerton, CA, USA) at 5000 x g for 30 min at 20^{o}C. The supernatants were carefully decanted and the tubes were inverted for 10 min to allow free drain. The tubes containing sediments were then weighed and the difference between the dry and wet weights was calculated as the water binding capacity.

3.3.6. Determination of Insoluble, Soluble, and Total Dietary Fiber Content

Insoluble, soluble, and total dietary fiber contents in washed and non-washed bran were measured according to the procedure in the Total Dietary Fiber Assay Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland). The kit contained three enzymes, thermostable α-amylase (3000 Ceralpha Units/mL), protease (350 Tyrosine Units/mL), and amyloglucosidase (3300 Units/mL of soluble starch), that were used to hydrolyze and depolymerize starch after it was gelatinized, solubilize and depolymerize proteins, and hydrolyze starch fragments to glucose, respectively. MES/TRIS buffer, 0.05M, was prepared by dissolving 19.52 g 2-(N-Morpholino) ethanesulfonic acid hydrate (MES) (M8250, Sigma-Aldrich, St. Louis, MO, USA) and 14.2 g TRIS (hydroxymethyl) aminomethane (T1503, Sigma-Aldrich, St. Louis, MO, USA) in 1.7 L deionized water; the pH was adjusted to pH 8.2 with 6.0 N NaOH. The buffer was then diluted to 2 L with deionized water and its pH adjusted to 8.3 at 20^{o}C. Other reagent grade chemicals used for total dietary fiber assay included ethanol (KOPTEC, King of Prussia, PA, USA), acetone (Sigma-Aldrich, St. Louis, MO, USA) and hydrochloric acid (EMD Chemicals Inc., Gibbstown, NJ, USA).

To determine insoluble, soluble, and total dietary fiber contents, approximately one gram of ground bran was weighed into a 400 mL tall-form beaker followed by addition of 40 mL of MES/TRIS buffer. The beakers were gently swirled until bran particles were completely dispersed in the buffer solution. 50 μL of heat-stable α-amylase were added to the beakers containing samples. The beakers were swirled, covered with aluminum foil, and incubated in a boiling water bath (Blue M, Blue Island, IL, USA) for 35 min with continuous shaking. After incubation with heat-stable α-amylase, the samples were cooled to 60^{o}C and any rings around beakers were scraped down with a spatula and rinsed with 10 mL of deionized water. To every sample, 100 μL

of protease were added before the samples were incubated for 30 min in a shaking water bath at 60^{o}C. The samples were then removed from the water bath and 5 mL of 0.561 N HCl were added to each sample to bring the pH from 4.1 to 4.8. After pH adjustment, the samples were subjected to 200 μL of amyloglucosidase and incubated for 30 min in a shaking water bath at 60^{o}C. After amyloglucosidase treatment, the samples were filtered through cleaned [Micro-90 concentrated cleaning solution (Z281506, Sigma-Aldrich, St. Louis, MO, USA)] Pyrex Gooch Crucibles (CLS329450, Sigma-Aldrich, St.Louis MO, USA) containing 0.5 g celite (C8656, Sigma-Aldrich, St. Louis, MO, USA). Each residue was washed twice with 10 mL of deionized water preheated to 70^{o}C, and the filtrate and water washings were saved for soluble dietary fiber analysis. Each residue was then washed twice with 10 mL of 95% ethanol and 10 mL acetone. The crucibles containing washed residues were dried overnight in the convection oven (Model 737F, Fisher Scientific, Itasca, IL, USA) at 103^{o}C. After drying, the samples were weighed and one was used to determine protein content while the other was incinerated to determine ash content. Percent insoluble dietary fiber was calculated using the formula stated in the Total Dietary Fiber Megazyme Kit.

Soluble dietary fiber was determined by adding four volumes of 95% ethanol preheated to 60^{o}C to filtrate and washings from the insoluble dietary fiber step and allowing soluble fiber to precipitate for 60 min at room temperature. The soluble fiber precipitate containing enzyme digest was filtered through crucibles containing celite. In sequence, the residue was washed twice with 15 mL of 78% ethanol, then twice with 15 mL of 95% ethanol, and finally twice with 15 ml acetone. The crucibles containing soluble fiber residues were dried overnight in the convection oven (Model 737F, Fisher Scientific, Itasca, IL, USA) at 103^{o}C. The percentage of soluble dietary

fiber was calculated using the formula stated in the Total Dietary Fiber Megazyme Kit. Total dietary fiber in washed and non-washed samples was calculated as the sum of insoluble dietary fiber plus soluble dietary fiber.

3.3.7. Proximate Compositions of Non-Washed and Washed Bran

Ash and moisture contents of washed and non-washed bran samples were determined according to AACCI Approved Methods 08-01 and 44-19, respectively (AACCI 2000). The protein contents in non-washed and washed bran were determined by Leco Nitrogen Combustion Analyzer (Model FP-2000, Leco Inc., St. Joseph, MI, USA). A factor of 5.7 was used to calculate crude protein. Total fat contents were determined using Soxhlet extraction apparatus according to AACCI Approved Method 30-25 with modifications. Samples were placed in 30 mm x 80 mm thimbles and extracted for 24 hr. Following extraction, petroleum ether was removed from the sample by a rotary evaporator. The fat remaining in round bottom flasks was dried at 100^{o}C in the convection oven (Model 737F, Fisher Scientific, Itasca, IL, USA) for 1 h.

3.3.8. Statistical Analysis

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure to determine significant differences among the samples. Means were compared using Fisher's Least Significant Difference (LSD) procedure. Significance was defined at the 5% level.

3.4. RESULTS AND DISCUSSION

3.4.1. Total Starch

Washing decreased total starch contents in NWB425 and NWB1000 by over 70% (Table 3.1). Total starch was significantly higher in NWB425 than in NWB1000, and in WB425 than in WB1000. The higher value for total starch content in NWB425 may be caused by flour that ends up in the bran fraction during the process of flour milling. Starchy endosperm that is loosely attached to the bran may also easily pass through the larger sieves (e.g., 1000 μm) during sifting and ultimately increase total starch content in NWB425. It is difficult to compare total starch results of washed bran to published data because this is the first study to look at washed wheat bran. Ralet et al (1990) and Xie et al (2008) measured total starch of native wheat bran and obtained 18.60% and 17.96%, respectively, which are within the range of total starch contents of both NWB425 and NWB1000 in the present study.

Table 3.1. Total Starch Composition* of Non-Washed and Washed Wheat Bran Samples

Bran Sample	Total Starch (%, w/w)
NWB425	23.41 ± 0.56a
NWB1000	14.43 ± 0.41b
WB425	5.52 ± 0.19c
WB1000	4.11 ± 0.16d

NWB425: Nonwashed bran ground to pass through a 425μm screen.
NWB1000: Nonwashed bran ground to pass through a 1000μm screen.
WB425: Washed bran ground to pass through a 425μm screen.
WB1000: Washed bran ground to pass through a 1000μm screen.
*Values followed by the same letter in the same column are not significantly different from each other ($p<0.05$).
Values are means of three determinations ± standard deviation.

3.4.2. Effect of Washing on the Thermal Properties of Non-Washed and Washed Wheat Bran Starch Samples

The onset transition temperatures (T_o) and peak transition temperatures (T_p) of washed bran samples (WB425 and WB1000) were significantly higher than those of non-washed bran samples (NWB425 and NWB1000), whereas the transition enthalpies (ΔH) of washed bran samples were significantly lower than those of the non-washed bran samples (Table 3.2). Goering and DeHaas (1972) and Seib (1994) pointed out that the onset and peak transition temperatures of starch granules are affected by the particle size distribution, with smaller particle starch granules (B-type starch) exhibiting lower gelatinization temperatures, whereas large particle size granules (A-type starch) exhibit higher gelatinization temperatures. However, in the present study, the differences in the gelatinization onset temperatures and gelatinization peak temperatures of non-washed and washed bran starch samples are thought to be caused by the differences in the water binding capacities among the bran samples (Table 3.3). The fact that washed wheat bran samples bind more water than non-washed bran samples may explain why the onset and peak transition temperatures of washed wheat bran starch were higher than those of non-washed wheat bran starch. It is possible that the ability of washed wheat bran to absorb more water reduces the water required for gelatinization of washed wheat bran starch, thereby increasing onset and peak transition temperatures. In the present study, the differences in the values of ΔH in non-washed and washed bran samples may be caused by different amounts of total starch present in these samples (Table 3.1). The higher the concentration of starch in the sample, the greater the energy required for gelatinization to take place, thereby increasing the values of ΔH. The lower values of total starch (Table 3.1) and transition enthalpy in washed bran samples indicate that the method

developed in the present study to wash away most of residual starchy endosperm from wheat bran was effective.

Table 3.2. Effect of Washing on the Thermal Properties* of Non-Washed and Washed

Sample**	To (^{o}C)	Tp (^{o}C)	ΔH (J/g)
NWB425	60.66 ± 0.14b	66.94 ± 0.13a	2.83 ± 0.25a
NWB1000	60.04 ± 0.09a	67.13 ± 0.23b	1.95 ± 0.18b
WB425	63.53 ± 0.02c	67.62 ± 0.18c	0.51 ± 0.01c
WB1000	64.22 ± 0.06d	67.63 ± 0.02c	0.53 ± 0.01c

*To: transition onset temperature; Tp: transition peak temperature; ΔH: transition enthalpy; values followed by the same letter in the same column are not significantly different from each other ($p<0.05$).

**For explanation of abbreviations, see Table 3.1.

Values are means of three determinations ± standard deviation.

3.4.3. Water Binding Capacity of Non-Washed and Washed Bran Samples

Water binding capacity (WBC) refers to the amount of water that a quantity of dry sample retains after centrifugation (Nelson 2001). The WBC was significantly greater for the larger particle size bran (Table 3.4). For the non-washed bran samples, the larger particle size (1000µm) had a WBC 31% greater than that of the smaller particle size (425µm) bran sample. The WBC of non-washed bran samples are consistent with those of Cadden (1987), Blackwood et al (2000), and Zang and Moore (1997) who reported increases in WBC with increasing wheat bran particle size. Washing increased the WBC of WB1000 by 28% and of WB425 by 51% relative to their counterpart NWB samples. However, WBC was not significantly affected by particle size of the washed wheat bran samples. The higher WBC of NWB1000 as compared to NWB425 was probably related to the presence of greater amounts of soluble dietary fiber in non-washed wheat bran (Table 3.3). Eastwood et al (1983) indicated that the manner in which various cereal fibers bind water is very important for stool bulking effect. Because insoluble fibers absorb water in the manner of a sponge (Oakenfull 2001), washing to remove starchy endosperm from the bran results in the exposure of previously blocked pores and sponge-like cell structures, which may enable the WB particles to hold more water. Robertson and Eastwood (1981) reported that fibers that loosely bind water increased stool weight whereas those that strongly bind water had little or no effect on stool weight.

Table 3. 3. Water Binding Capacity (WBC)* of Non-Washed and Washed Wheat Bran

Bran Sample**	WBC (g of water/1g of dry sample)
NWB425	2.67 ± 0.07c
NWB1000	3.90 ± 0.04b
WB425	5.47 ± 0.07a
WB1000	5.43 ± 0.20a

*Values followed by the same letter in the same column are not significantly different from each other ($p<0.05$).
**For explanation of abbreviations, see Table 3.1.
Values are means of three determinations.

3.4.4. Insoluble Dietary Fiber, Soluble Dietary Fiber and Total Dietary Fiber

The insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and total dietary fiber (TDF) contents of NWB425, NWB1000, WB425, and WB1000 are listed in Table 3.4. The dietary fiber contents of NWB425 and NWB1000 are in the range of those reported by Kahol et al (2000), Nandini and Silimath (2001), Esposito et al (2005), Bilgicli et al (2007), and Claye et al (1996). There is no published data available for comparison of dietary fiber contents of washed bran, since this is the first study to determine dietary fiber contents of WB. The IDF content in NWB1000 was 21.5% higher than in NWB425. Also, larger particle size (1000µm) had a 21% higher contents of SDF than that of the smaller particle size (425µm). IDF content was significantly higher in WB425 and WB1000 than in NWB425 and NWB1000 by 42.3% and 27.3%, respectively. SDF contents were also significantly reduced by washing. SDF was 50.5% lower in WB425 compared to NWB425, whereas SDF was decreased by 65.9% in WB1000 relative to NWB1000. The increase in IDF contents in the washed bran samples was mainly due to the loss of starch, water-soluble proteins, and other soluble polysaccharides during washing. Significant decreases in SDF contents in washed wheat bran compared to non-washed bran indicate that the residual starch still adherent to wheat bran after roller milling contains significant amounts of soluble fiber. These results are in agreement with Dexter and Wood (1996) who reported variations in insoluble and soluble dietary fiber contents in different layers of the wheat kernel. Understanding how dietary fiber varies in different layers can help in developing processing techniques that can alter physicochemical properties and the proportion of insoluble and soluble dietary fiber in wheat bran (Dexter and Wood 1996).

Table 3.4. Dietary Fiber Composition* of Non-Washed and Washed Wheat Bran samples

Bran Sample**	Insoluble Dietary Fiber (%,w/w)	Soluble Dietary Fiber (%, w/w)	Total Dietary Fiber (%, w/w)
NWB425	38.77 ± 0.28c	3.90 ± 0.16b	42.67 ± 0.12c
NWB1000	49.40 ± 0.14b	4.93 ± 0.01a	55.33 ± 0.20b
WB425	67.25 ± 0.21a	1.93 ± 0.05c	69.18 ± 0.23a
WB1000	68.00 ± 1.27a	1.68 ± 0.09c	69.68 ± 0.75a

*Values followed by the same letter in the same column are not significantly different from each other ($p<0.05$).

**For explanation of abbreviations, see Table 3.1.

Values are means of four determinations ± standard deviation.

3.4.5. Proximate Compositions (dry weight basis) of Non-Washed and Washed Wheat Bran

Washing decreased protein content from 14.7% in NWB425 to 11.5% in WB425 and from 15.7% in NWB1000 to 13.6% in WB1000 (Table 3.5). It is clear from the results that washing of milled bran resulted in a net decrease in protein. The decrease in protein content in washed bran samples suggests that some protein was removed along with residual endosperm adherent to wheat bran during the process of washing. However, it is important to understand that most of the protein in washed bran comes from non-protein nitrogen. In general, fat contents were neither significantly different for NWB425 and WB425 nor for NWB1000 and WB1000. Fat contents in washed and non-washed bran samples are consistent with findings by other authors (Caprez et al 1986; Gualberto et al 1997). However, fat content was higher in NWB1000 and WB1000 than in NWB425 and WB425. It is possible that large particle sized bran contains more of the germ portion of wheat than the smaller particle sized bran. Further studies would be needed to confirm this phenomenon.

In general, the ash contents were significantly different among the NWB425, WB425, NWB1000, and WB1000 samples, but significantly higher in the larger particle sized bran samples. Washing significantly affected ash contents in wheat bran samples. Ash content increased from 4.48% in NWB425 to 5.38% in WB425 and from 6.73% in NWB1000 to 6.98% in WB1000. The lower ash contents in NWB425 and NWB1000 may be attributed to higher amounts of flour in these bran samples (i.e., a dilution effect). The NWB ash values in the present study are comparable to those reported by Shenoy and Prakash (2002) and Gualberto et al (1997).

Table 3.5. Proximate Composition* of Non-Washed and Washed Bran Samples

Bran sample**	Moisture (%, w/w)	Protein (%, w/w)	Fat (%, w/w)	Ash (%, w/w)
NWB425	2.89 ± 0.04a	14.70 ± 0.02b	4.65 ± 0.04ab	4.48 ± 0.01d
NWB1000	3.15 ± 0.21b	15.67 ± 0.15a	5.19 ± 0.03a	6.73 ± 0.03b
WB425	3.00 ± 0.05b	11.50 ± 0.08c	3.78 ± 0.06b	5.38 ± 0.03c
WB1000	2.86 ± 0.05a	13.58 ± 0.08d	5.16 ± 0.08a	6.98 ± 0.02a

*Values followed by the same letter in the same column are not significantly different from each other ($p<0.05$).

**For explanation of abbreviations, see Table 3.1.

Values are means of three determinations ± standard deviation.

3.5. CONCLUSIONS

The washing method described in this removed a significant amount of starchy endosperm adherent to wheat bran after milling. Washing removes starchy endosperm, thereby modifying thermal properties of the washed bran components. Washing significantly changed the contents of IDF and SDF. Particle size was not associated with significant differences in TDF, IDF and SDF contents of washed wheat bran. The water binding capacity of NWB425 was significantly lower than that of NWB1000. Water binding capacities of WB425 and WB1000 were not significantly different from each other, but significantly higher than those of their counterpart non-washed bran samples. Overall, the results from this study indicate that the presence of residual starchy endosperm on milled wheat bran can easily interfere with analyses to determine the bran samples' physicochemical properties and composition.

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CHAPTER 4

EFFECTS OF PARTICLE SIZE COUPLED WITH EXTRUSION PROCESSING CONDITIONS ON DIETARY FIBER PROFILE OF NON-WASHED AND WASHED WHEAT BRAN

4.1. ABSTRACT

Wheat bran, a by-product of roller milling during the milling of wheat, contains substantial amounts of residual starch that may interfere with the analysis of its dietary fiber profile. Therefore, the specific objective of the present study was to develop a method (washing) of removing most of the starch adherent to milled wheat bran and to investigate the effects of extrusion processing conditions on the contents of soluble dietary fiber and insoluble dietary fiber in non-washed bran (NWB) and washed bran (WB). NWB and WB samples obtained from milled soft white wheat were ground to pass through 1000 or 425 μm screens and extruded through a co-rotating and inter-meshing twin-screw extruder under conditions of varying screw configurations (low and high shear), feed moisture (25 and 35%), screw speed (100 and 400 rpm), and die temperature (100 and 150^{o}C). The results of the present study indicate that for NWB of both particle sizes, extrusion processing conditions slightly increased soluble dietary fiber content, but decreased insoluble dietary fiber content. Certain extrusion processing conditions slightly increased soluble dietary fiber in both washed bran samples. The insoluble dietary fiber contents of extrudates made from the larger particle size WB were significantly lower than those of the counterpart non-extruded WB.

4.2. INTRODUCTION

The role of dietary fiber in human nutrition cannot be overlooked. In recent years, dietary fiber has attracted significant attention due to health care officials' and nutritionists' recommendendations that consumption of dietary fiber can help to maintain good health (McCleary 2007). Dietary fiber reduces the risk of cardiovascular disease, certain forms of cancer, and alleviates constipation (Park et al 2005). Dietary fibers are grouped into insoluble and soluble dietary fibers and each plays a different role in human health. Insoluble dietary fiber is important for proper bowel function and may reduce symptoms of chronic constipation, diverticular disease, and hemorrhoids, while soluble dietary fiber is associated with the reduction of cholesterol levels and attenuation of blood glucose (Schaafsma 2004).

Wheat bran, which comprises approximately 15% of the whole wheat kernel, is rich in dietary fiber. Like any other plant cell wall, wheat bran is composed of cellulose, hemicelluloses, pectin, and other associated components such as lignin, cutin, and suberin (Devries et al 1999). Wheat bran is obtained as a by-product of roller milling during the milling process of wheat grain. It is used to make "all bran" extruded breakfast cereal (Eastman et al 2001) besides being used by the baking industry as the major source of dietary fiber (Vetter 1988). Although wheat bran is rich in dietary fiber, the soluble dietary fiber content is lower than that of insoluble dietary fiber. The range of soluble dietary fiber content in wheat bran is 1.5 to 4.0% (w/w) whereas that of insoluble dietary fiber is 35 to 48% (w/w) (Kahol and Chow 2000; Nandini and Silimath 2001; Esposito et al 2005). The dietary fiber profile of wheat bran depends on the source of wheat and the milling technique used.

Extrusion cooking technology, a common processing technique to produce breakfast cereals and a variety of ready-to-eat foods including breakfast cereals, snacks, baby foods, and

meat analogs (Vasanthan et al 2002), has been used by a number of investigators to study its effects on the dietary fiber profiles of high-fiber ingredients including wheat bran (Asp 1986; Theander and Westerlund 1987; Aloe et al 1989; Camire et al 1990). The high temperature, shear and other variables employed during extrusion processing are believed to alter the architecture of cell wall polysaccharides (Selvendra and Robertson 1994; Nyman et al 1994). The effect of extrusion cooking conditions on the dietary fiber profile in wheat bran has been investigated by a number of researchers. Ralet et al (1990), Wang and Klopfenstein (1993), and Gaulberto et al (1997) showed that extrusion processing conditions cause disintegration of insoluble dietary fiber linkages, leading to an increase in soluble dietary fiber. On the other hand, Varo et al (1983) reported that heat treatment had no effect on dietary fiber distribution in wheat flour.

Previous studies have used non-washed (native) wheat bran to study the effects of extrusion cooking variables on its dietary fiber profile. However, wheat bran obtained upon roller milling of wheat has a substantial amount of residual endosperm still adherent to it. During extrusion cooking, it is possible that the residual endosperm adherent to wheat bran undergoes certain chemical modifications and interferes with the analyses that are subsequently used to determine the dietary fiber profile in extruded wheat bran. Therefore, the objective of the present study was to investigate the effects of bran particle size coupled with extrusion processing conditions on the contents of soluble dietary fiber and insoluble dietary fiber in both non-washed and washed wheat bran.

4.3. MATERIALS AND METHODS

4.3.1. Wheat Bran Sample

The non-washed wheat bran sample used in this study was milled from soft white wheat of the 2009 crop and supplied by Star of the West Milling Company (Frankenmuth, MI, USA). The sample was stored at 4^{O}C until the studies were conducted.

4.3.2. Preparation of Washed Wheat Bran

Washing of wheat bran was performed according to section 3.3.2 of this dissertation.

4.3.3. Grinding and Sifting

Dried Non-Washed Bran (NWB) and Washed Bran (WB) samples were ground according to section 3.3.3 of this dissertation.

4.3.4. Extrusion of Bran Samples

Washed and non-washed wheat bran samples, sifted and collected sequentially through 1000µm and 425µm screens, were extruded in duplicate on a co-rotating and inter-meshing twin-screw extruder (Model MP 19T2-25, APV Baker, Grand Rapids, MI, U.S.A.) with a 19-mm barrel diameter and a barrel length to diameter ratio (L/D) of 25:1. A twin-screw extruder feeder system (K-TRON, Pittman, NJ, U.S.A.) was used to feed bran samples at 2 kg/hr through the hopper into the extruder barrel. The extruder barrel was divided into five heating zones with zone 1 closest to the feed port and zone 5 at the die exit end. The extruder and the feeder were equipped with control panels that displayed all the processing conditions, such as temperature for each of the five heating zones, screw speed, pressure, torque, and feed rate. The Brook Crompton

E2 Metripump (Hudders Field, England) was used to inject water inside the extruder barrel that resulted in the desired moisture content during extrusion processing of wheat bran samples.

The effect of extrusion processing variables on the contents of soluble dietary fiber and insoluble dietary fiber was investigated using a 2 x 2 x 2 x 2 x 2 complete factorial design (Table 4.1), with two levels of bran particle size (425 μm and 1000 μm), two screw speeds (100 and 400 rpm), two temperatures (100 and 150^{o}C), two moisture levels (25 and 35%), and two screw configurations (low and high). The low and high shear screw configurations used in the present study are illustrated in Table 4.2 and extrusion processing conditions (moisture, screw speed, and barrel temperature profile) are depicted in Table 4.3. The low shear screw configuration consists of more conveying screw elements whereas the high shear screw configuration consists of more mixing screw elements. Extruded samples were dried in a convection oven overnight at 60^{o}C. Grinding of extruded samples was accomplished using a Udy Cyclone mill (Udy Corp., Fort Collins, CO) equipped with a 0.5 mm screen. The ground extruded samples were stored in "Ziplock" bags at 4^{o}C until further analysis.

Table 4. 1 . Experimental Design

Independent Variables	Factors
Bran Treatment	washed, non-washed
Bran Particle Size (μm)	1000, 425
Extruder Shear Configuration*	low, high
Extruder Temperature (ºC)	100, 150
Feed Moisture Content (%)	25, 35
Extruder Screw Speed (rpm)	100, 400

*See Table 4.2.

Table 4.2. Screw Elements Used for Low and High Shear Extrusion Configurations

Low Shear Configuration	High Shear Configuration
8 D Twin Lead Screws	8 D Twin Lead Screws
7 x 30^{o} Forward Kneading Elements	7 x 30^{o} Forward Kneading Elements
8 D Twin Lead Screws	8 D Twin Lead Screws
3 x 60^{o} Forward Kneading Elements	4 x 60^{o} Forward Kneading Elements
3 x 30^{o} Reverse Kneading Elements	4 x 30^{o} Reverse Kneading Elements
2 D Single Lead Screws	2 D Twin Lead Screws
4 x 60^{o} Forward Kneading Elements	6 x 60^{o} Forward Kneading Elements
3 x 30^{o} Reverse Kneading Elements	4 x 30 Reverse Kneading Elements
2 D Single Lead Screws	1 D Single Lead Screws
	7 x 90^{o} Kneading Elements
	2 D Single Lead Screws

D: screw diameter (= 19 mm).
One kneading element = 0.25 D.

Table 4.3. Extrusion Cooking Conditions* for NWB425, NWB1000, WB425, and WB1000

Feed Moisture (% wb)	Screw Speed (rpm)	Barrel Temperature (^{o}C) for Zones 1-5**
25	100	40/60/80/100/100
25	400	40/60/80/100/100
35	100	40/60/80/100/100
35	400	40/60/80/100/100
25	100	40/60/100/140/150
25	400	40/60/100/140/150
35	100	40/60/100/140/150
35	400	40/60/100/140/150

*Same for low and high shear screw configurations (see Table 4.2).
**Zone 5 is nearest the exit die.
NWB425: Nonwashed bran ground to pass through a 425μm screen.
NWB1000: Nonwashed bran ground to pass through a 1000μm screen.
WB425: Washed bran ground to pass through a 425μm screen.
WB1000: Washed bran ground to pass through a 1000μm screen.

4.3.5. Determination of Soluble Dietary Fiber and Insoluble Dietary Fiber in Non-Washed and Washed Wheat Bran

Total dietary fiber content in washed and non-washed bran was measured according to the procedure in the Total Dietary Fiber Assay Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland) and modified as stated in section 3.3.5 of this dissertation.

4.3.7. Statistical Analysis

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA.). Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure to determine significant differences among the samples. Means were compared using Fisher's Least Significant Difference (LSD) procedure. Significance was defined at the 5% level.

4.4. RESULTS AND DISCUSSION

4.4.1. Effect of Bran Particle Size Coupled with Extrusion Processing Conditions Under Low Shear Screw Configuration on the Contents of Insoluble Dietary Fiber (dry weight basis) in Extrudates Made from Non-Washed and Washed Wheat Bran

The insoluble dietary fiber contents in non-extruded NWB425 and NWB1000 were 38.8% (w/w) and 49.4% (w/w), respectively (Table 4.4). The lower value of insoluble dietary fiber in the NWB425 before extrusion may be attributed to the presence of proportionately more starch compared with that in NWB1000 (Table 3.1). Extrusion processing slightly decreased the contents of insoluble dietary fiber in extrudates made from NWB425 for most extruded conditions studied, except for the sample extruded at 150-25-400 (Table 4.4). The insoluble fiber contents in all extruded NWB1000 samples were lower than that in the non-extruded NWB1000 sample. The contents of insoluble dietary fiber in extrudates made from NWB1000 were not statistically significantly different from each other, except for the sample extruded at 150-35-400, which had the lowest amount of insoluble dietary fiber (44.4%, w/w) (Table 4.4). The slight decreases in the insoluble dietary fiber contents in the present study are in agreement with results of previous investigators (Ralet et al 1990; Lue et al 1991; Wang et al 1993; Quaglia et al 1995; Gualberto et al 1997) who reported loss of insoluble dietary fiber in wheat bran, upon extrusion processing. These authors suggested that the decrease in the insoluble dietary fiber during extrusion was caused by high shear of the rotating screws. However, contradictory results have also been reported. Siljestrom et al (1986), Asp and Bjorck (1989), Wang et al (1993) and Artz et al (1990) reported that extrusion processing did not have an effect on the contents of insoluble and soluble dietary fibers in wheat bran. In the present study, the largest decreases in insoluble dietary fiber were from 38.8% for NWB425 to 37.3% in ENWB425 (a 4% decrease) and from 49.4% for

NWB1000 to 44.4% in ENWB1000 (a 10% decrease) under extrusion conditions listed in Table 4.4.

The effects of extrusion processing conditions on the contents of insoluble dietary fiber in extrudates made from WB425 and WB1000 are shown in Table 4.5. The insoluble dietary fiber contents in WB425 were minimally affected by extrusion processing conditions. However, certain extrusion conditions resulted in very minor increases in the contents of insoluble dietary fiber in WB425 extrudates. Björck (1984) and Camire et al (1990) reported that one of the possible mechanisms by which insoluble dietary fiber increases during extrusion is the formation of polysaccharide-lipid complexes which could not be hydrolyzed by the enzymes used in the enzymatic-gravimetric procedure of dietary fiber analysis, and thus measure as insoluble dietary fiber. Vitagoliane et al (2008) suggested three ways through which extrusion processing may cause increases in insoluble dietary fiber: by (1) starch retrogradation, (2) formation of protein-polysaccharide complexes, and (3) oxidation of dietary fiber and phenolic compounds. Some of these mechanisms were suggested for samples containing substantial amounts of starch. It is not clear if the same mechanisms are exactly applicable to WB425, which had a total starch content of not more than 5.52%, w/w (Table 3.1, Chapter 3). The increases in insoluble dietary fiber contents in WB425 extrudates were very small; hence, it is difficult to conclude that these increases were due to chemical reactions as described by previous authors. Unlike WB425, which was virtually unaffected by extrusion conditions, the contents of insoluble dietary fiber in extrudates from WB1000 were significantly lower compared to non-extruded WB1000 (Table 4.5). However, the insoluble dietary fiber contents were not significantly different among the extruded samples. Since an extruder is considered a form of a grinder, it is possible that larger bran particles are easily mechanically broken by screw shear during extrusion, thereby decreasing

the contents of insoluble dietary fiber. On the other hand, the small bran particles in WB425 may not be as easily affected by extrusion shear. This probably explains why the contents of the insoluble dietary fiber in extrudates from WB425 were not significantly decreased by low shear extrusion processing.

Table 4.4. Effects of Extrusion Cooking Conditions under Low Shear Screw Configuration on the Contents of Insoluble and Soluble Dietary Fibers* of Extrudates Made from NWB425 and NWB1000 Samples**

	NWB425		NWB1000	
Extrusion Conditions***	IDF (% w/w)	SDF (% w/w)	IDF (% w/w)	SDF (% w/w)
Non-extruded	38.8 ± 0.3b	3.9 ± 0.2a	49.4 ± 0.1b	4.9 ± 0.1a
100-25-100	37.3 ± 0.7a	5.0 ± 0.3bc	45.6 ± 1.2ab	5.9 ± 0.1bc
100-25-400	37.5 ± 0.3a	4.6 ± 0.2abc	46.1 ± 1.9ab	6.1 ± 0.4c
100-35-100	37.3 ± 0.5a	4.7 ± 0.2abc	45.7 ± 1.0ab	5.8 ± 0.6abc
100-35-400	38.3 ± 0.3a	5.5 ± 0.6c	46.1 ± 1.0ab	5.9 ± 0.2bc
150-25-100	37.3 ± 0.3a	4.8 ± 0.2ab	46.7 ± 1.1ab	6.3 ± 0.1c
150-25-400	39.1 ± 0.3b	4.2 ± 0.5b	44.7 ± 1.6ab	6.0 ± 0.2c
150-35-100	37.3 ± 0.0a	4.5 ± 0.4abc	45.4 ± 1.6ab	5.7 ± 0.7abc
150-35-400	37.3 ± 0.0a	5.0 ± 0.6bc	44.4 ± 1.1a	5.1 ± 0.4ab

*Values followed by the same letter in the same column are not significantly different from each other ($p<0.05$).
**IDF: insoluble dietary fiber; SDF: soluble dietary fiber; for NWB425 and NWB1000, see Table 4.3.
***100-25-100 (temperature, degrees C – moisture, % wet basis – screw speed, rpm).
Values are means of four determinations ± standard deviation.

4.4.2. Effect of Bran Particle Size and Extrusion Processing Conditions Under Low Shear Screw Configuration on the Contents of Soluble Dietary Fiber (dry weight basis) in Extrudates Made from Non-Washed and Washed Wheat Bran

The contents of soluble dietary fiber in NWB425 and NWB1000 before extrusion were 3.90% and 4.94%, respectively (Table 4.4). The lower value of soluble dietary fiber in NWB425 compared with that in NWB1000 may be attributed to the presence of more residual starchy endosperm in NWB425, which was susceptible to hydrolysis by the α-amylase used in the total dietary fiber determination protocol. The physical interaction between residual endosperm and the bran may explain the differences in soluble dietary fiber contents in NWB425 and NWB1000. Most of the residual endosperm in NWB425 was in the form of discrete flour particles whereas that in NWB1000 was adhered to bran tissue. It is possible that the residual endosperm adherent to NWB1000 is higher in soluble dietary fiber than that in the NWB425 sample. Dexter and Wood (1996) reported that soluble dietary fiber content distribution varies in different layers of the wheat kernel. The results of the present study indicated that soluble dietary fiber content was higher in the residual starchy endosperm adherent to the bran of milled wheat.

The soluble dietary fiber contents in most of the extruded NWB425 and NWB1000 samples were higher than in their respective non-extruded samples. The largest percent increase in soluble dietary fiber with low shear extrusion was from 3.90% to 5.50% (a 29% increase) for NWB425, and from 4.9% to 6.3% (a 22% increase) for NWB1000; the largest increase in soluble dietary fiber in NWB425 was obtained when the sample was extruded at the highest temperature (150^{o}C), lowest moisture (25%), and lowest screw speed (100 rpm), whereas that in NWB1000 was obtained when the sample was extruded at the lowest temperature (100^{o}C), highest moisture (35^{o}C), and highest screw speed (400 rpm). These samples were both extruded under low shear

screw configuration. The results of soluble dietary fiber in the NWB425 and NWB1000 upon low shear extrusion are comparable to those reported in literature (Caprez et al 1986; Aoe et al 1989; Ralet et al 1990; Wang et al 1993; Gaulberto et al 1997). Several investigators have suggested that the increase in the contents of soluble dietary fiber in extruded samples may be caused by breakage of insoluble dietary fiber, thereby forming small fragments that are soluble in the aqueous ethanol that is used in the total dietary fiber determination procedure (Camire et al 1990; Gaulberto et al 1997; Guillon and Champ 2000; Vasanthan et al 2002; Singh et al 2007; Vitagoliane et al 2008). According to the results of soluble dietary fiber obtained from the present study, the amount (on a weight per weight basis) by which soluble dietary fiber increased was higher than the amount of insoluble dietary fiber lost during extrusion (Table 4.4.). It is also important to note that even for the extrusion condition (150-25-400) that did not change the amount of insoluble dietary fiber in NWB425 and NWB1000 samples, soluble dietary fiber was still increased by 7.4%. Therefore, the notion should be questioned that increased soluble dietary fiber is brought about by breakdown of insoluble dietary fiber. These results suggest that insoluble dietary fiber breakdown is not the only mechanism by which soluble dietary fiber increases during extrusion processing. It is possible that the residual endosperm, still adherent to wheat bran after the milling process, undergoes certain chemical modifications during extrusion that result in chemical components that are resistant to hydrolysis by enzymes used in the dietary fiber quantification procedure. This is in agreement with Theander and Westerlund (1987) who pointed out that extrusion cooking generates 1, 6-anhydrosaccharides that react with starch and starch-containing products to form new branched glucans that are resistant to α-amylase hydrolysis.

Unlike NWB425 and NWB1000, for which all studied low shear extrusion processing conditions increased soluble dietary fiber, low shear extrusion of WB425 and WB1000 samples resulted in either increases or decreases in the quantity of soluble dietary fiber (Tables 4.5). Although the contents of insoluble dietary fiber in all extruded WB1000 samples were significantly lower than in non-extruded WB1000, the contents of soluble dietary fiber in WB1000 extrudates were either higher or lower than non-extruded WB1000. Again, this implies that the increase in soluble dietary fiber during extrusion processing is not caused merely by redistribution of insoluble dietary fiber. It is difficult to compare results from the washed bran samples in this study with those in published literature which mainly focus on non-washed wheat bran. It is unknown if the cause of the variation in soluble dietary fiber contents of extruded washed bran samples was due to extrusion processing conditions or whether this reflects limitations of the current procedure for the determination of dietary fiber, i.e., that the procedure may not be sensitive enough for samples containing very small amounts of soluble dietary fiber.

Table 4.5. Effects of Extrusion Processing Conditions under Low Shear Screw Configuration on the Contents of Insoluble and Soluble Dietary* Fibers of Extrudates Made from WB425 and WB1000 Samples**

	WB425		WB1000	
Extrusion conditions***	IDF (% w/w)	SDF (% w/w)	IDF (% w/w)	SDF (% w/w)
Non-extruded	67.3 ± 0.2a	1.93 ± 0.01de	68.0 ± 1.3a	1.68 ± 0.10bcd
100-25-100	68.6 ± 0.5ab	1.96 ± 0.04de	62.4 ± 0.6b	2.16 ± 0.21e
100-25-400	68.4 ± 0.8ab	2.28 ± 0.04f	63.5 ± 1.7b	1.72 ± 0.16cd
100-35-100	67.6 ± 0.2a	1.73 ± 0.21cd	63.3 ± 0.5b	1.39 ± 0.16abc
100-35-400	68.5 ± 0.3ab	1.92 ± 0.20de	64.0 ± 0.9b	1.91 ± 0.01de
150-25-100	69.7 ± 0.1b	1.60 ± 0.04bc	63.6 ± 1.7b	1.22 ± 0.16a
150-25-400	69.3 ± 1.1b	2.05 ± 0.04ef	63.4 ± 0.3b	1.37 ± 0.11ab
150-35-100	67.5 ± 0.4a	1.28 ± 0.11a	63.5 ± 0.8b	1.28 ± 0.18a
150-35-400	67.5 ± 0.4ab	1.42 ± 0.13ab	63.0 ± 0.2a	1.92 ± 0.12de

*Values followed by the same letter in the same column are not significantly different from each other ($p<0.05$).
**See Table 4.4.
***For explanations of abbreviations, see Tables 4.3 and 4.4.
Values are means of four determinations ± standard deviation.

4.4.3. Effect of Bran Particle Size and Extrusion Processing Conditions Under Low and High Shear Screw Configurations on the Contents of Insoluble and Soluble Dietary Fibers in Extrudates Made from WB425 and WB1000

No relationship was found between either low or high shear screw configuration and the contents of insoluble or soluble dietary fiber of the extruded samples studied (Figs. 4.1 and 4.2). Certain extrusion conditions with high shear configurations, increased or decreased the soluble dietary fiber and insoluble dietary fiber in both non-washed and washed wheat bran. Wang et al (1993) studied the effect of screw speed during extrusion of non-washed wheat bran milled from hard red winter wheat. In that study, wheat bran was extruded at low screw speed (100 rpm), medium screw speed (300 rpm), and high screw speed (400 rpm). They reported that the insoluble dietary fiber content was significantly decreased from 53.7% (non-extruded wheat bran) to 50.2% in extrudates made from wheat bran extruded at the highest screw speed (400 rpm). Under the same condition, the soluble dietary fiber content significantly increased from 1.72% to 4.25%. In another study, Gualberto et al (1997) investigated the effect of screw speed (50, 70, and 100 rpm) on the contents of insoluble and soluble dietary fiber in non-washed wheat bran milled from a Canadian hard red wheat. They reported a slight decrease in insoluble dietary fiber in bran extrudates extruded at 100 rpm. However, they reported a significant increase in soluble dietary fiber under the same extrusion condition.

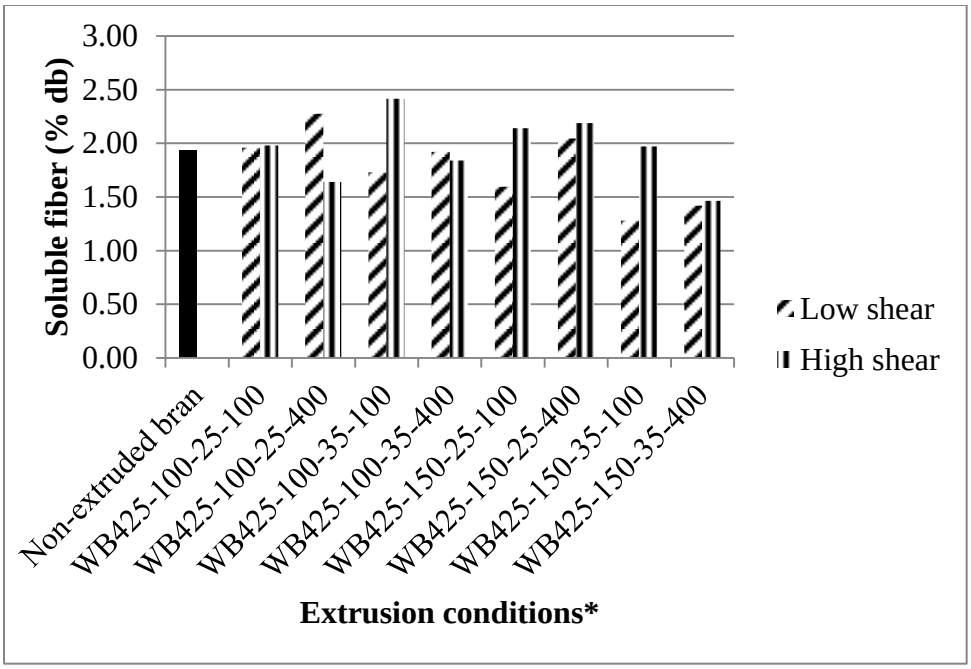

Figure 4.1. Effects of low and high shear screw configurations (see Table 4.2 for details) on the contents of soluble dietary fiber in washed wheat bran extrudates.

*For abbreviations, see Tables 4.3 and 4.4.

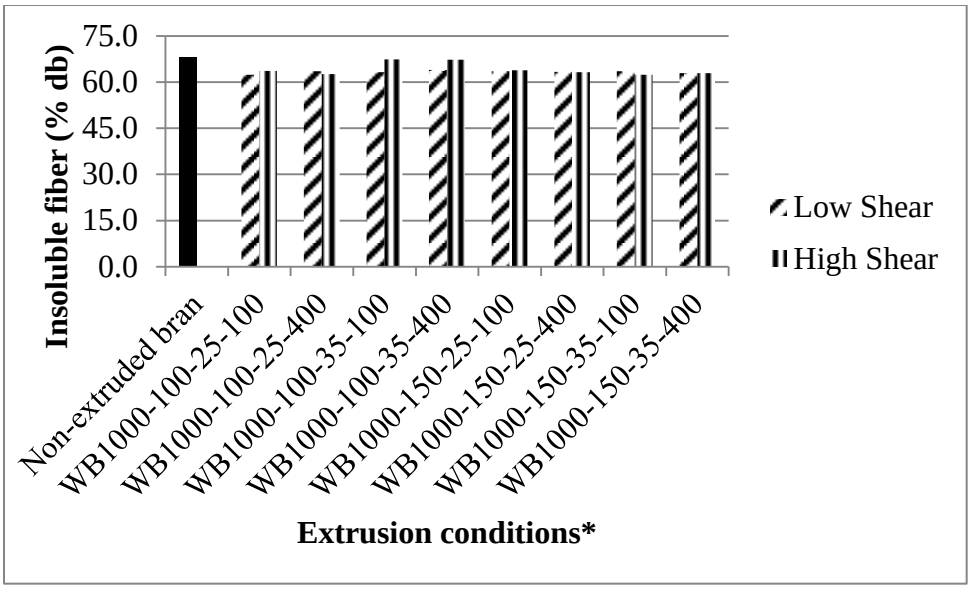

Figure 4.2. Effect of low and high shear screw configurations (see Table 4.2 for details) on the contents of insoluble dietary fiber in WB1000 extrudates.

*For abbreviations, see Tables 4.3 and 4.4.

4.5. CONCLUSIONS

Extrusion increased the quantity of soluble dietary fiber in both NWB425 and NWB1000 samples. Most of the extrusion cooking conditions studied demonstrated slight decreases in insoluble dietary fiber of both NWB425 and NWB1000. Certain extrusion processing conditions slightly increased the soluble dietary fiber in both WB425 and WB1000. The current protocol for determining dietary fiber content may not be sensitive enough to detect very small amounts of dietary fiber.

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LITERATURE CITED

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CHAPTER 5

EFFECTS OF EXTRUSION PROCESSING ON THE PHYSICOCHEMICAL PROPERTIES OF NON-WASHED AND WASHED WHEAT BRAN

5.1. ABSTRACT

The effects of extrusion processing on water binding capacity of bran, molecular weight of soluble fiber, thermal degradation properties of bran samples, and *in vitro* binding of bile acids of non-washed and washed wheat bran samples were studied. Extrusion processing increased the water binding capacity of non-washed bran samples but did not have an effect on the water binding capacity of washed bran samples. The molecular weights of soluble dietary fibers extracted from non-washed bran samples were significantly higher than those of soluble dietary fibers extracted from washed bran samples. Extrusion further decreased the molecular weight of soluble fiber in all wheat bran samples. The onset degradation temperature and peak degradation temperature of non-washed bran were higher than those of washed bran samples. However, degradation enthalpy of washed bran was significantly higher than that of non-washed bran. Extrusion did not affect degradation properties of wheat bran samples. *In vitro* binding of bile acids was slightly higher in washed bran samples compared to non-washed bran samples. The increased bile acid binding by samples with very low soluble dietary fiber suggests that binding of bile acids in wheat bran may be due to mechanisms other than binding by soluble dietary fiber.

5.2. INTRODUCTION

Extrusion cooking is a high-temperature short-time (HTST) processing technique that is used to produce a variety of food products such as ready-to-eat breakfast cereals, texturized vegetable protein, pet foods, instant powders, baby foods, expanded products and pasta products (Ficarella et al 2006; Singh and Singh 2004; Jin et al 1995; Vasanthan 2002). During extrusion, the sample ingredients are mixed and sheared by rotating screws inside a heated barrel. There is a possibility that extrusion cooking can result in molecular and structural changes of extruded food ingredients including wheat bran. These extrusion-induced changes may affect the physicochemical properties of extruded high-fiber ingredients as well as physiological properties (Margareta and Nyman 2003).

The high demand for healthy foods by consumers has prompted the food industry to increase the amount of insoluble and soluble dietary fibers in processed food products (Chawla and Patil 2010). Consumption of foods high in dietary fibers from different sources has been shown to reduce risks of cardiovascular disease, obesity (Alfieri et al 1995; Haworth et al 2005), diverticulitis and hemorrhoids (Gordon 1989; Brown et al 1999; Park et al 2005; Gutkoski et al 2007), and result in effective stool bulking and reduced transit time (Cummings 1997).

Wheat bran is used by the baking industry to increase the amount of dietary fiber in baked products. It is very important to know that the ratio of soluble dietary fiber to insoluble dietary fiber in native wheat bran is very low. The risk of cardiovascular disease is decreased with highly viscous soluble dietary fiber due to its ability to reduce cholesterol in the serum (Park et al 2005). Ralet et al (1990), Wang and Klopfenstein (1993), and Gaulberto et al (1997) reported that extrusion cooking increased the amounts of soluble dietary in wheat bran. However, it is not clear

if the soluble dietary fiber created by this process has the same bile acid binding properties as the original soluble dietary fiber.

Bile acids are synthesized from cholesterol in the liver and used in the digestion of lipids. After aiding in lipid digestion, more than 90% of bile acids are reabsorbed back to the liver by enterohepatic circulation (Hoffman 1977), where they are reutilized. Binding of bile acids by cholesterol-lowering drugs and soluble fibers in the duodenum are some of the ways that may lead to reduction of blood cholesterol, especially of low-density lipoproteins, the type known to increase the risk of cardiovascular disease (Eastwood and Hamilton 1968; Kritchevsky and Story 1974).

To understand the role of processing on the physiological properties of fibers, it is important to evaluate the physicochemical properties, such as molecular weight, water binding capacity, and binding of bile acids. Functional properties of polymers including dietary fibers are affected by their molecular weights. For example, polymers that are more viscous, in general have higher molecular weights (Shibanuma et al 1996). High performance size exclusion liquid chromatography equipped with multi-angle laser-light scattering and refractive index is a powerful tool that has been widely used to determine absolute molecular weight of soluble polymers (Aberle et al 1994; Fishman et al 1996).

Processing that involves cooking has been reported to decrease molecular weight of soluble polysaccharides. Snavberg et al (1995) reported that molecular weight of soluble fiber in carrots was significantly lower following different types of processing. It is possible that processing may result in the de-polymerization of fiber polysaccharides. Bjork et al (1984) and Nyman et al (1987) pointed out that breakage of glycosidic linkages may facilitate fermentation by the beneficial microorganisms in the colon. However, de-polymerization of soluble fiber

polysaccharides may negatively affect their nutritional properties (Albersheim et al 1960). Cleavage of fiber polysaccharides results in a decreased viscosity that may lower their ability to alter lipid metabolism. Jenkins et al (1978) pointed out that cleavage of glycosidic linkages of soluble polysaccharides may decrease their ability to regulate glucose metabolism. Ralet et al (1991) investigated the effects of extrusion cooking conditions on the water binding capacity of wheat bran. They reported that extrusion cooking increased the ability of wheat bran to bind more water compared to non-extruded wheat bran. The ability of fiber to improve bowel movement and to undergo fermentation in the colon mainly depends on their water binding properties.

No research has been conducted to understand how particle size and extrusion cooking conditions affect physicochemical properties of wheat bran that has been washed to remove most of the residual starchy endosperm. Therefore, the overall objective of the present study was to evaluate the effects of particle size coupled with extrusion cooking conditions on water binding capacity, thermal degradation, molecular weight, and binding of bile acids of extruded non-washed and washed wheat bran.

5.3. MATERIALS AND METHODS

5.3.1. Raw Material

The wheat bran used in this study was milled from soft white wheat of the 2009 crop and supplied by the Star of the West Milling Company (Frankenmuth, MI, U.S.A).

5.3.2. Preparation of Washed Wheat Bran

Preparation of washed wheat bran is described in section 3.3.2 of this dissertation. Non-washed and washed wheat bran samples were stored at 4^{o}C until extrusion studies were conducted.

5.3.3. Grinding and Sifting of Wheat Bran Samples

Dried non-washed bran (NWB) and washed bran (WB) samples were ground and sifted according to section 3.3.3 of this dissertation.

5.3.4. Extrusion of Wheat Bran

Extrusion of wheat bran samples is described in section 4.3.4 of this dissertation.

5.3.5. *In vitro* Binding of Bile Acids

In vitro binding of bile acids in wheat bran samples was determined according to the method of Camire et al (1993) as modified by Kahlon and Chow (2000). Briefly, conjugated bile acids: glycochenodeoxycholic acid, glycocholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid, and taurocholic acid (CBA-1KT, Sigma-Aldrich, St. Louis, MO, USA) were formulated based on composition of human bile to form a stock solution. To simulate acidic

gastric digestion, 100 mg of wheat bran samples were weighed into 17 mL-screw capped tubes and 1 mL of 0.01N HCl was added to each test tube. The samples were incubated for 1 hr in a shaking water bath maintained at 37^{o}C. After this acidic digestion, the pH was adjusted to 6.3 by addition of 0.1 mL 0f 0.1N NaOH. To simulate conditions in the small intestine, 4 mL of bile acid mixture and 5 mL of pancreatin solution (providing amylase, protease and lipase for digestion of samples) were added to each sample and incubated for 1 h at 37^{o}C under continuous agitation. Samples were transferred to 10- mL Oak Ridge centrifuge tubes and centrifuged (Model J2-21M, Beckman Instruments Inc., Fullerton, CA, USA) at 33,000 x g for 20 min at 25^{o}C. Concentration of bile acids in the supernatant was determined enzymatically according to the Bile Acid Kit No. 450-A (Trinity Biotech, Bray, Wicklow, Ireland).

5.3.6. Determination of Weight-Average Molecular Weight of Soluble Fiber Using High-Performance Size Exclusion Liquid Chromatography

The soluble fiber powder used for determining weight-average molecular weight was prepared according to the procedure described in section 3.3.4 of this dissertation. The weight-average molecular weights of soluble fibers from non-washed, washed, extruded non-washed and extruded washed bran samples were determined using high performance size exclusion liquid chromatography (HPSELC, Agilent 1200, Agilent Technologies, Santa Clara, CA, USA). HPSELC analyses were conducted using an Ohpak SB-804 HQ column (Shoko America Inc., CO, USA) with packing materials having pore diameters of 10 µm. A Wyatt miniDawn TREOS multi-angle laser light scattering detector (Wyatt Tech. Corp., Santa Barbara, CA, USA) and a Wyatt Optilab refractive index detector (Wyatt Tech. Corp., Santa Barbara, CA, USA) were used

to measure light scattering and refractive index values, respectively. The laser sources of these detectors were operated at 658 nm. Each sample of soluble fiber powders was mixed with deionized water (2.5 mg/mL) and filtered through a 0.2 μm syringe filter prior to injection. The HPSELC analyses of the soluble fiber isolated from bran samples were performed at 70^{O}C for 30 min, with sample concentrations of 2.5 mg/mL and eluent flow rates of 0.5 mL/ min. De-ionized water was used as the solvent. The data processing was performed using the ASTRA 5.3.4.18 software package (Wyatt Tech. Corp., Santa Barbara, CA, USA)

5.3.7. Thermal Decomposition Properties of Wheat Bran Samples

Thermal decomposition properties studies of non-washed, washed, extruded non-washed, and extruded washed wheat bran samples were studied by a Differential Scanning Calorimetry (DSC) (Model Q100 V9.9 Build 303, Greifensee, Switzerland). Each sample was weighed into a DSC aluminum pan and heated and from 20 to 400^{O}C at the heating rate of 10^{O}C/min. A sealed empty pan was used as a reference. Onset decomposition temperature (T_o), peak decomposition temperature (T_p), and decomposition enthalpy (ΔH) were recorded and analyzed using DSC software (Universal V4. 7A, TA Instruments, Newcastle, DE, USA).

5.3.8. Water Binding Capacity

Water binding capacity was determined according to section 3.3.5 of this dissertation.

5.3.9. Statistical Analysis

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA.). Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure to determine significant differences among the samples. Means were compared using Fisher's Least Significant Difference (LSD) procedure. Significance was defined at the 5% level.

5.4. RESULTS AND DISCUSSION

5.4.1. Water Binding Capacity

Table 5.1 shows the effects of particle size coupled with extrusion cooking conditions (low shear screw configuration, temperature, moisture, and screw speed) on the water binding capacity of washed and non-washed bran samples. Among the non-extruded wheat bran samples, the water binding capacity of NWB1000 was significantly higher than that of NWB425. The results of water binding capacities of NWB425 and NWB1000 samples from the present study are consistent with those reported by others (Kirwani 1974; Connell 1976; Heller et al 1977; Kimura 1977), who reported higher water binding capacity for the large particle size non-washed bran. They reported that the inability of fine wheat bran to bind more water, as compared to large particle size bran, may be attributed to lower contents of hydrophilic hemicelluloses in small particle size fibers. Ralet et al (1990) reported that water binding capacity of wheat bran increases with increases in insoluble fiber. In the present study, the insoluble dietary content in NWB1000 was higher than that in NWB425 (Table 3.3). The water binding capacities of washed bran samples were significantly higher than those of non-washed bran samples before extrusion (Table 3.4). Contrary to non-washed bran, water binding capacities of washed bran samples were not significantly affected by particle size. The possible explanation for this phenomenon is that removal of starch by washing aids in the exposure of wheat bran interstices, which are easily filled up with water. The water binding capacity of washed bran samples cannot be compared to published literature, since this is the first study to look at water binding capacity of washed bran.

Extrusion cooking conditions significantly increased the water binding capacity in extruded NWB425 and NWB1000. However, there were no significant differences among the individual extrusion conditions. The increases in water binding capacities in extrudates from

NWB425 and NWB1000 may be due to the presence of partially gelatinized starch. Anderson and Ng (2003) pointed out that the ability of starch to bind water was influenced by extrusion processing conditions. The water binding capacities of extruded WB425 and WB1000, which were lower in starch than the NWB samples, were not affected by the extrusion cooking conditions studied.

The water binding capacity of a particular fiber is an indicator of its impact in the gastrointestinal tract. Water binding can be affected by the chemical composition and physical properties of fibers (Guillon and Champ 2000). Thibault et al (1992) indicated that water binding capacity of fibers can be influenced by the way fibers are processed, for example, by grinding and extrusion cooking. In the present study, the water binding capacity of the bran samples was affected by particle size, washing, and extrusion.

Table 5. 1. Effects of Extrusion Conditions under Low Shear Screw Configuration on the Water Binding Capacities of NWB425, NWB1000, WB425, and WB1000 Samples*

Extrusion Conditions**	Water Binding Capacity			
	NWB425	NWB1000	WB425	WB1000
Non-extruded	2.67 ± 0.07a	3.89 ± 0.04a	5.47 ± 0.07abc	5.43 ± 0.02b
100-25-100	5.51 ± 0.07bc	4.89 ± 0.03e	5.66 ± 0.13bc	5.48 ± 0.04b
100-25-400	5.61 ± 0.16d	4.38 ± 0.04b	5.83 ± 0.02c	5.39 ± 0.03ab
100-35-100	5.50 ± 0.14bc	4.71 ± 0.09d	5.76 ± 0.07c	5.40 ± 0.18b
100-35-400	5.61 ± 0.01d	4.45 ± 0.11bc	5.84 ± 0.10c	5.27 ± 0.02ab
150-25-100	5.54 ± 0.01bc	4.56 ± 0.07cd	5.38 ± 0.04ab	5.86 ± 0.06c
150-25-400	5.34 ± 0.04b	4.46 ± 0.04bc	5.64 ± 0.03bc	5.33 ± 0.14ab
150-25-100	5.48 ± 0.08bc	4.94 ± 0.10e	5.25 ± 0.05a	5.45 ± 0.01b
100-25-400	5.43 ± 0.06bc	4.34 ± 0.08b	5.20 ± 0.02a	5.50 ± 0.05b

*Values followed by the same letter in the same column are not significantly different from each other ($p<0.05$).
NWB425: Nonwashed bran ground to pass through a 425µm screen.
NWB1000: Nonwashed bran ground to pass through a 1000µm screen.
WB425: Washed bran ground to pass through a 425µm screen.
WB1000: Washed bran ground to pass through a 1000µm screen.
**100-25-100 (temperature, degrees C – moisture, % wet basis – screw speed, rpm).
Values are means of three determinations ± standard deviation.

5.4.2. Effect of Washing, Bran Particle Size and Extrusion on Weight-Average Molecular Weights of Soluble Fibers Isolated from Wheat Bran Samples

The weight average molecular weights of soluble fibers isolated from NWB425 and NWB1000 were significantly higher than those of soluble fibers isolated from WB425 and WB1000 (Table 5.2). The weight average molecular weights of soluble fibers isolated from extruded NWB425 (ENWB425), ENWB1000, EWB425, and EWB1000 were lower than those of soluble fibers isolated from their respective counterpart non-washed bran samples (Table 5.2). The lower weight average molecular weights of soluble fibers isolated from washed bran samples may be due to the loss of soluble polymers, such as starch, during the washing process. This is confirmed by comparison of the total starch contents in the non-washed and washed bran samples (Table 3.1). Snavberg et al (1997) studied the effect of microwave cooking on soluble fiber in green beans and found that the weight average molecular weight decreased after microwave treatment. It is possible the extrusion processing conditions break down fiber glycosidic linkages, resulting in de-polymerization of fiber (Margaretta and Nyman 2003). In another study, Svanberg et al (1995) reported a decrease in weight average molecular weight of soluble fiber in carrots following blanching and boiling. The lower weight average molecular weights of soluble fibers isolated from extruded non-washed and extruded washed bran samples may be due to extrusion conditions, such as high temperature and shear, which may have led to depolymerization of soluble polymers. Decrease in weight average molecular weight has nutritional significance. Lowering the weight average molecular weight of soluble polymers, such as soluble dietary fiber, may impair their ability to modulate glucose and hormonal responses in the blood (Gustafsson et al 1995).

Table 5. 2. Effect of Washing, Particle Size, and Extrusion on the Weigh Average Molecular Weight* of Soluble Dietary Fiber in Wheat Bran Samples

Sample*	Weight Average Molecular Weight ($x10^5$)
NWB425	751.0
NWB1000	390.0
WB425	28.3
WB1000	23.6
ENWB425	70.6
ENWB1000	13.7
EWB425	2.2
EWB1000	2.2

*E: Extruded; for all other abbreviations, see Table 5.1.

5.4.3. Thermal Decomposition of Wheat Bran Samples

Differential Scanning Calorimetry (DSC) is a very important technique that is used to study thermal properties of polymers. The onset decomposition temperature (T_o), peak decomposition temperature (T_p), and decomposition enthalpy values (ΔH) of non-washed, washed, extruded non-washed and extruded washed bran samples are depicted in Table 5.3. From the results, it is clear that the onset decomposition temperature (T_o) of NWB1000 was significantly higher than that of WB1000. Extrusion cooking did not have an effect on the T_o of the samples in this study. The T_p values of NWB and ENWB were not significantly different from each other. Likewise, the T_p values of WB and EWB did not significantly differ from each other. However, T_p values of WB and EWB were significantly higher than those of NWB and ENWB, respectively. The decomposition enthalpies of NWB and ENWB were significantly lower than those of WB and EWB. The higher decomposition enthalpy values for WB and EWB indicate that more energy is required to decompose washed wheat bran. Based on the fact that the amount of insoluble dietary fiber is significantly higher in WB samples than in NWB samples (Table 3.3), it could be speculated that there is proportionately more cellulose in washed wheat bran than in non-washed wheat bran (weight per weight); cellulose requires substantial amounts of energy to decompose. The thermal properties of non-washed, washed, extruded non-washed and extruded washed bran samples cannot easily be compared to previous studies because of limited reports in the literature on this topic.

Table 5.3. Thermal Decomposition Properties* of Non-Washed Bran, Washed Bran, Extruded Non-Washed Bran, and Extruded Washed Bran Samples

Sample**	To (^{o}C)	Tp (^{o}C)	ΔH (w/g)
NWB1000	257.8 ± 0.2b	285.8 ± 1.4b	102.8 ± 0.3a
WB1000	250.9 ± 2.8a	279.5 ± 0.3a	140.6 ± 0.1b
ENWB1000	257.2 ± 0.1b	287.3 ± 0.1b	102.6 ± 0.3a
EWB1000	250.2 ± 1.4a	277.2 ± 0.1a	131.3 ± 1.4c

*To: onset decomposition temperature; Tp: peak decomposition temperature; ΔH: decomposition enthalpy; values followed by the same letter in the same column are not significantly different from each other ($p<0.05$).

**For explanation of abbreviations, see Tables 5.1 and 5.2.

Values are means of three determinations ± standard deviation.

5.4.4. Binding of Bile Acids by Wheat Bran Samples

Cholestyramine significantly bound more bile acids than any of the wheat bran samples (Table 5.4). Assigning a bile acid binding of 100% to cholestyramine, the relative binding of bile acids by NWB425, WB425, NWB1000, WB1000, ENWB425, EWB425, ENWB1000, and EWB1000 were 18.09, 20.31, 19.20, 23.53, 17.09, 22.42, 18.65, and 25.64%, respectively. It is clear from the results that bile acid binding was higher in washed wheat bran compared to non-washed bran. Particle size did not have an effect on the binding of bile acids. Extrusion did not significantly affect binding of bile acids either.

Two mechanisms have been proposed to explain the ability of dietary fibers to decrease cholesterol in the blood. The first mechanism is that increased amounts of propionate are produced during microbial fermentation of these fibers and that the propionate inhibits cholesterol biosynthesis (Anderson et al 1990). The second proposed mechanism is that the presence of highly viscous fibers in the ileum interferes with bile acid re-absorption (Marlett 1997). Zhang et al (1992) and Lund et al (1989) reported that increased loss of bile acids from the body may be attributed to the higher intestinal bacterial count with diets high in fermentable fiber, because bacteria may bind bile acids in the colon. Marcus and Heaton (1986) and Alberts et al (1996) reported that wheat bran reduces intestinal transit time and binds bile acids, thereby decreasing the risks of colon cancer.

According to the results from the present study, washed bran samples bound more bile acids than non-washed bran samples. The increased bile acid binding by washed bran samples, which are lower in soluble dietary fiber but higher insoluble dietary fiber (Table 3.3), indicate that binding of bile acids by wheat bran may be due to mechanisms other than binding by soluble dietary fiber. These results are consistent with those reported by Kahlon and Chow (2000). They

studied *in vitro* binding of bile acids by rice bran, oat bran, wheat bran, and corn bran and reported that rice and wheat brans significantly bound more bile acids than oat fiber, even though oat bran is higher in soluble dietary fiber than rice bran and wheat bran. The soluble dietary fiber in oat bran has been linked to reduction in serum cholesterol.

Table 5.4. *In Vitro* Binding of Bile Acids by Wheat Bran Samples

Sample*	Bile acid binding**	Binding relative to cholestyramine (%)
Cholestyramine	9.01 ± 0.07c	100.00c
NWB425	1.63 ± 0.14ab	18.09ab
NWB1000	1.73 ± 0.14ab	19.20ab
WB425	1.83 ± 0.27ab	20.31ab
WB1000	2.12 ± 0.27ab	23.53ab
ENWB425	1.54 ± 0.27a	17.09a
EWB425	1.68 ± 0.14ab	18.65ab
ENWB1000	2.02 ± 0.11ab	22.42ab
EWB1000	2.31 ± 0.22b	25.64b

*For explanation of abbreviations, see Tables 5.1 and 5.2.

**Values are means of three determinations ± standard deviation.

5.5. CONCLUSIONS

The extrusion cooking conditions utilized in the present study increased the water binding capacities of NWB425 and NWB1000. Extrusion cooking had no effect on water-binding capacities of washed bran samples. The weight average molecular weights of WB samples were lower than those of NWB samples. Extrusion processing decreased weight average molecular weights of soluble fiber in both non-washed and washed bran samples. Thermal decomposition properties of wheat bran samples were not affected by extrusion cooking. Washing did affect thermal decomposition of wheat bran samples. *In vitro* binding of bile acids was higher in washed bran than in non-washed bran. Binding of bile acids was found to be higher in extrudates from WB425 and WB1000. Overall, *in vitro* binding of bile acids was higher for the larger bran particle size samples, for both non-extruded and extruded samples.

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CHAPTER 6

EFFECT OF TREATED WHEAT BRAN ON THE BAKING PROPERTIES OF BREAD AND COOKIES

6.1. ABSTRACT

The ability of dietary fibers to provide health benefits has prompted the food industry to develop a variety of fiber-enriched foods including baked products. The purpose of this study was to investigate the effects of adding non-washed, washed, extruded non-washed, and extruded washed wheat bran samples on (1) the Farinograph results and pasting properties of a hard wheat flour and a soft wheat flour, (2) the quality properties of bread and cookies substituted with 5% or 15% bran sample, and (3) the dietary fiber profile in bread samples. Farinograph water absorptions of each of the flour samples substituted with wheat bran samples were higher than that of the respective hard wheat flour, and increased with the increase in wheat bran substitution level. Pasting properties obtained from the Rapid Visco Analyzer indicated that peak, trough, breakdown, and final viscosities of hard and soft wheat flour samples containing wheat bran were lower than those of the respective hard and soft wheat flours. Bread formulations containing wheat bran resulted in breads with increased loaf weights and decreased loaf volumes. Substituting soft wheat flour with 5% or 15% bran significantly decreased cookie spread. Insoluble and soluble dietary fiber distributions were affected by baking conditions. The contents of insoluble dietary fiber in the crumb and crusts of breads baked from hard wheat flour and hard wheat flour substituted with various bran samples were significantly higher than those in the control hard flour. On the other hand, the contents of soluble dietary fiber in the crumb and crusts of breads baked with hard wheat flour substituted with wheat bran were not significantly different from each other.

6.2. INTRODUCTION

Baked products are a major source of revenue in the United States. Fresh bread and cookies alone generated 11 billion dollars in 2010 (AIB 2011). Bread, a commonly consumed baked product all over the world, is made from hard wheat flour in addition to yeast, salt, and water. Other ingredients that may be included in the formula are fat, sugar, milk, oxidants, enzymes, surfactants, and other additives to increase the taste, quality, and shelf life of the bread. It is important to know that flour is the major ingredient in the bread formulation. The proteins in the flour, when water is added, form a visco-elastic dough that retains gas and results in the formation of bread. Yeast is also an important ingredient in the bread formulation. It converts fermentable carbohydrates into carbon dioxide and ethanol, and these gases are responsible for the production of a leavened loaf of bread. Salt is usually added to bread formulations as a source of taste as well as an improver of dough rheological properties. Another important ingredient is water, which acts as a plasticizer and solvent. Without water, there would be no dough and therefore no viscous flow properties and many of the reactions that normally take place during fermentation could not occur.

Cookies are different from bread because they are made from soft wheat flour. In addition, cookie formulations are high in sugar and fat and low in water. Cookies are popular baked products because they are convenient and have extended shelf life (Singh and Mohamed 2007).

Wheat is a major ingredient of staple foods, such as breads and noodles for people all over the world (Posner 2000) and is a good source of protein, vitamins, and minerals besides being an excellent source of dietary fiber. The positive impact of dietary fiber on human health has prompted the food industry to develop foods enriched with dietary fiber. Dietary fiber is

incorporated in a variety of foods, including baked products, to improve their nutritional benefit. Wheat bran is one of the major sources of dietary fiber incorporated into baked products. Wheat bran has the ability to provide numerous health benefits in the gastrointestinal tract (Anderson et al 1994). Insoluble dietary fiber in wheat bran is more effective in preventing constipation than that found in fruits and vegetables (Anderson et al 1994). Consumption of wheat bran significantly increases stool weight and volume (Balasubramanian et al 1987; Jenkins 1987; Lampe et al 1993) and may possibly reduce the risk of colon cancer (National Research Council 1989; Kroon et al 1997; Cho and Clark 2001).

The challenge encountered by food technologists is that incorporation of wheat bran into food systems deteriorates overall quality properties of end-use products (Guillon and Champ 2000). Extrusion may modify physicochemical properties of wheat bran and reduce its deteriorative effects and result in improved quality attributes in processed foods, for example of baked products, which could ultimately increase human fiber consumption. The main purposes of the present study were to investigate the effects of adding non-washed, washed, extruded non-washed and extruded washed wheat bran samples on (1) the Farinograph results and pasting properties of a hard wheat flour and a soft wheat flour, (2) the quality properties of bread and cookies substituted with 5% or 15% of the bran samples, and (3) the amounts of insoluble dietary fiber and soluble dietary fiber in wheat flour and in the crust and crumb of baked bread.

6.3. MATERIALS AND METHODS

6.3.1. Materials

Hard wheat flour was obtained from the Mennel Milling Co. (Fostoria, OH, U.S.A). Soft wheat flour and wheat bran were obtained from the Star of the West Milling Co. (Frankenmuth, MI, U.S.A).

6.3.2. Preparation of Washed Bran

Wheat bran was washed according to the procedure described in section 3.3.2 in this dissertation.

6.3.3. Grinding and Sifting

Dried Non-washed Bran (NWB) and Washed Bran (WB) samples were ground according to section 3.3.3 of this dissertation.

6.3.4. Preparation of Extruded Wheat Bran

Extruded wheat bran samples were extruded according to section 4.3.4 of this dissertation.

6.3.5. Farinograph Properties

Farinograph properties of hard wheat flour samples containing 0%, 5% (w/w) or 15% (w/w) of non-washed, washed, extruded non-washed, or extruded washed wheat bran were determined using a 50-g mixing bowl Farinograph according to the AACCI Approved Method 54-21 (AACCI 2000). Values of water absorption, dough development time, stability, and mixing tolerance index were obtained from the Farinograms.

6.3.6. Pasting Properties

The pasting properties of hard and soft wheat flour samples containing 0%, 5% (w/w) or 15% (w/w) of non-washed, washed, extruded non-washed, or extruded washed bran were determined using a Rapid Visco Analyzer (RVA Model 4, Newport Scientific Inc., Warriewood, Australia). Three grams (at a 14% moisture basis) of each sample were mixed with 25 mL of distilled water in an RVA cup and mixed in the RVA heating block. Pasting properties of samples were analyzed using Standard 1 profile according to the AACCI Approved Method 76-21 (AACCI 2000). Pasting data such as peak viscosity (highest viscosity during heating), trough viscosity (lowest viscosity following peak viscosity), breakdown viscosity (peak viscosity minus trough viscosity), setback (final viscosity minus peak viscosity), and final viscosity (viscosity at the end of heating cycle) were processed and obtained by the Thermocline version 1.2 Software (Newport Scientific Inc., Warriewood, Australia).

6.3.7. Breadmaking

Hard wheat flour (100 g at 14% moisture) was used to make control bread. Breads were also prepared from wheat flour substituted with 5% (w/w) or 15% (w/w) of non-washed bran, washed bran, extruded non-washed or extruded washed bran samples. Breadmaking was performed according to the optimized straight-dough breadmaking procedure (AACCI Approved Method 10-10B; AACCI 2000) with some modifications. In addition to flour and bran, the bread formulation contained sugar (6.0 g), salt (1.5 g), shortening (3.0 g), yeast (1.96 g, Safmex, Milwaukee, WI, USA), ascorbic acid (5.0 mg), and water. The amount of water in the formulation was calculated based on the Farinograph water absorption. Breads were baked in a rotary oven

(National MFG Co., Lincoln, NE, USA) at 215^{o}C for 24 min. After baking, breads were removed from the baking pans and allowed to cool at room temperature for 2 hr before quality properties were determined.

6.3.8. Determination of Bread Quality Properties

After cooling, the breads were weighed and their volumes determined by the rapeseed displacement method. Loaves of bread were then stored in sealed Ziplock bags at room temperature for 24 hr prior to bread firmness determination. Using a slicer (Model FF-3199, Hobart MFG. Co. Troy, OH, USA), the loaves were cut to obtain two bread slices (each 12.5 mm thick) from the center of the loaf which were used for firmness analysis. Bread firmness was determined by compression using a texture analyzer (Model TA-XT2, Texture Technologies, Corp., Scarsdale, NY, USA) equipped with a 25 kg load cell. The force (N) required to compress 25% (6.25 mm) of two stacked slices of bread was determined with an acryl probe (38 mm diameter) at a compression rate of 1.77 mm/s. After determining bread firmness, the same two slices were stored in sealed 1 gal Ziplock bags at -20^{o}C until dietary fiber analysis.

6.3.9. Cookiemaking

Cookies from soft wheat flour and soft wheat flour substituted with 5% (w/w) or 15% (w/w) of non-washed bran, washed bran, extruded non-washed or extruded washed bran samples were prepared according to the AACC Method 10-54 (AACCI 2000) for Micro Wire-Cut Cookies. Cookie doughs were baked in a rotary oven (National MFG Co., Lincoln, NE, USA) for

11 min at 215^{o}C. Baked cookies were placed on a wire rack and cooled for 30 min at room temperature before determination of quality properties.

6.3.10. Determination of Cookie Quality Properties

The cookie quality properties evaluated after 30 min cooling time included weight, diameter, and thickness. Cookies were then placed in Ziplock bags and stored for 24 hr before cookie hardness was determined. Cookie hardness was determined using a texture analyzer (Model TA-XT2, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 25 kg load cell. The cookie was cut into two pieces by a blade (3 mm thickness) at a compression rate of 2.0 mm/s, and the maximum force (N) was recorded.

6.3.11. Determination of Insoluble and Soluble Dietary Fibers in Bread Crumb and Crust

The frozen slices of bread were thawed for 12 hr before crumb and crust samples were collected. The crumb that was used in this study was taken 2 cm from the edge of the bread slice. The crust used was 1 cm from the edge of the bread slice. The crumb and crust were torn into pieces by hand, placed in aluminum bowls and dried at 50^{o}C for 18 hr in the conventional oven (Model 737F, Fisher Scientific, Itasca, IL, USA). The dried pieces of crumb and crust were then crushed by hand prior to grinding in a Udy Cyclone Mill (Udy Corp., Fort Collins, CO, U.S.A) equipped with a 0.5 mm screen. The ground samples were stored in "Ziplock" bags at 4^{o}C until insoluble and soluble dietary fibers determination.

Insoluble and soluble dietary fibers in bread crumb and crust were determined according to section 3.3.4 of this dissertation with a modification. The weights of the dried insoluble dietary

fiber and soluble dietary fiber residues were designated as the weights of insoluble dietary fiber and soluble dietary fiber, respectively. No corrections for ash and protein were made due to the small amounts of each fiber in the samples.

6.3.12. Statistical analysis

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA.). Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure to determine significant differences among the samples. Means were compared using Fisher's Least Significant Difference (LSD) procedure. Significance was defined at the 5% level.

6.4. RESULTS AND DISCUSSION

6.4.1. Farinograph

The Farinograph properties of hard wheat flour (control) and hard wheat flour samples substituted with 5% (w/w) or 15% (w/w) bran are listed in Table 6.1. All the hard wheat samples that substituted with wheat bran had higher water absorption values than that of hard wheat flour (control). Farinograph water absorption increased significantly when bran substitution was increased to 15% (w/w). Water absorption was slightly higher for all samples containing washed bran as compared to the counterpart non-washed bran. The higher values of water absorption in hard wheat flour substituted with washed bran may be due to the fact that washed bran samples bind more water than non-washed bran samples (see water binding capacities of non-washed and washed bran samples listed in Table 3.3, Chapter 3). These results are consistent with those of Lang et al (1990) who reported that increasing the amount of high-fiber ingredients in wheat flour increases Farinograph water absorption. The dough development times of wheat flour samples substituted with 5% wheat bran samples were not significantly different from that of the control flour. However, with 15% bran-substitution, the dough development times were significantly different from that of the control flour and those of the flour samples substituted with 5% bran. It is possible that the higher amounts of fiber particles in the 15% bran-substituted samples interfered to greater extents with the hydration of flour proteins and starch by competing for water (Nelson 2001). The Farinograph stabilities of all flour samples substituted with 5% or 15% wheat bran samples were significantly lower than that of the control flour (Table 6.1). The lowest stability was found with flour sample substituted with 15% washed wheat bran. Stability is one of the dough mixing properties that indicates dough strength, with higher stabilities indicating stronger doughs and vice-versa. The decrease in Farinograph stability upon addition of cereal

brans has been reported by other investigators. Laurikainen et al (1998) reported a decrease in stability of the dough made from hard wheat flour substituted with 5% rye bran. The highest mixing tolerance index was displayed by hard wheat flour substituted with 15% washed bran. The increase in mixing tolerance indices for samples substituted with wheat bran was also reported by Zhang and Moore (1997) and Shenoy and Prakash (2002), although these authors utilized non-washed bran.

Table 6.1. Farinograph Properties of Hard Wheat Flour Substituted with Bran*

Sample	Water Absorption (%)	Dough Development Time (min)	Stability (min)	Mixing Tolerance Index (BU)
HWF	59.0 ± 0.3a	5.5 ± 0.0b	16.0 ± 0.7f	20.0 ± 0.0a
HWF-5NWB	61.2 ± 0.3b	5.5 ± 0.1b	13.5 ± 0.3d	20.0 ± 0.0a
HWF-5WB	62.2 ± 0.1c	5.0 ± 0.0a	10.0 ± 0.1b	30.0 ± 10.0b
HWF-5ENWB	61.0 ± 0.6b	5.0 ± 0.3a	15.0 ± 0.3e	20.0 ± 10.0a
HWF-5EWB	62.8 ± 0.1d	5.5 ± 0.1b	12.0 ± 0.3c	30.0 ± 0.0b
HWF-15NWB	66.2 ± 0.2e	6.0 ± 0.1c	12.0 ± 0.3c	20.0 ± 0.0a
HWF-15WB	69.9 ± 0.1g	6.5 ± 0.3d	9.0 ± 0.4a	50.0 ± 10.0c
HWF-15ENWB	67.4 ± 0.3f	6.5 ± 0.7d	12.0 ± 0.1c	20.0 ± 0.0a
HWF-15EWB	70.2 ± 0.3h	7.5 ± 0.1e	12.0 ± 0.3c	30.0 ± 0.0b

*Values followed by the same letter in the same column are not significantly different ($p<0.05$). HWF: hard wheat flour; NWB: non-washed bran; WB: washed bran; ENWB: extruded non-washed bran; EWB: extruded washed bran; 5 and 15 are bran substitution levels (%, w/w). Values are means of two determinations ± standard deviation.

6.4.2. Pasting Properties

The pasting data indicate that soft wheat flour had higher peak, breakdown, trough, setback and final viscosities than hard wheat flour (Table 6.2). These results are consistent with those of Ragaee and Abdel-Aal (2006). Both soft wheat flour and hard wheat flour samples containing 5% or 15% wheat bran had lower peak viscosities than the respective control flour samples. The decrease in peak viscosity in bran-substituted samples is likely due to a starch diluting effect.

Understanding how high-fiber ingredients such as wheat bran affect pasting properties of hard and soft wheat flours is very important since starch plays important roles during the manufacture of baked products, e.g., bread and/or other processes in which starch is heated in the presence of sufficient amounts of water. During heating, starch granules imbibe water, which increases viscosity of the food system, a process known as gelatinization. The increase in viscosity during heating of starch and other processes, such as cooling, are responsible for product structure, e.g., bread crumb (Artwell 2001; Couvain and Young 2006).

Table 6.2. Pasting Properties of Hard Wheat Flour and Soft Wheat Flour Substituted with Wheat Bran*

Sample	Peak Viscosity (RVU)	Trough Viscosity (RVU)	Breakdown Viscosity (RVU)	Final Viscosity (RVU)	Setback (RVU)
HWF	184.4	98.8	67.2	224.4	125.6
HWF-5NWB	151.5	101.1	50.4	218.8	117.8
HWF-15NWB	121.9	78.9	43.0	193.8	114.9
HWF-5WB	165.4	105.6	59.8	221.5	115.9
HWF-15WB	119.2	78.7	41.2	173.2	94.6
HWF-5ENWB	145.7	95.7	50.0	196.1	100.4
HWF-15ENWB	115.2	79.6	35.6	172.3	92.7
HWF-5EWB	117.6	82.2	35.3	168.5	86.2
HWF-15EWB	113.8	77.0	36.8	160.8	83.8
SWF	196.0	127.8	85.6	270.8	142.0
SWF-5NWB	109.2	56.3	52.8	143.3	87.0
SWF-15NWB	108.3	60.3	48.1	156.8	96.6
SWF-5WB	125.8	64.5	61.3	156.3	91.8
SWF-15WB	86.7	45.7	41.0	115.5	69.8
SWF-5ENWB	140.5	72.5	60.5	172.2	99.8
SWF-15ENWB	98.5	53.8	44.8	135.7	81.9
SWF-5EWB	130.8	74.9	55.8	165.2	90.2
SWF-15EWB	105.8	54.3	51.4	136.6	82.5

*SWF: Soft Wheat Flour; for all other abbreviations refer to Table 6.1.

6.4.3. Bread Quality Properties

Bread formulations containing 5% or 15% bran samples resulted in significantly lower loaf volumes than bread made from control flour (Table 6.3). There were no significant differences in bread volumes among breads made from flour samples substituted with 5% of non-washed, washed, extruded non-washed, or extruded washed bran. The loaf volumes of bread samples made from flour samples substituted with 15% WB were significantly lower than those substituted with 15% NWB. The bread volumes of bread samples made from flour samples substituted with 15% ENWB were significantly lower than those made from flour samples substituted 15% NWB. Breads made from hard wheat flour substituted with 15% EWB had the lowest bread volumes.

Addition of various high-fiber ingredients in bread formulations has been reported to decrease loaf volume (Hamid and Luan 2000; Pomeranz et al 1977; Dalgetty and Baik 2006; Hung et al 2007). Results obtained in the present study are consistent with those of Dalgetty and Baik (2006). Pomeranz et al (1977) reported that reduction in bread loaf volume in formulations with added fiber ingredients was caused by gluten dilution that ultimately reduces gas retention. Mujoo and Ng (2003) reported that addition of immature wheat meal (i.e., proportionately high in fiber) to the bread formulation decreased loaf volume. They suggested that reduction in loaf volume was probably due to reduced gas retention. In the present study, it can be speculated that the decrease in loaf volumes of breads made from flour substituted with 15% WB and 15% EWB, as compared to their counterpart NWB and ENWB samples, is due to their higher water binding capacities, (Table 5.1, Chapter 5 of this dissertation). It is possible that the presence of washed and extruded-washed wheat bran in the dough samples compete for water with flour proteins gliadins and glutenins, and impair formation of gluten network, which is responsible for gas

retention during fermentation and baking. In addition to gluten dilution caused by direct replacement of flour, the presence of wheat bran in the system may interfere with the development of the gluten network, thus impairing the ability of the dough to retain gas. Increasing the concentration of wheat bran in bread formulation increased bread weight. This could be caused by the ability of wheat bran to bind more water than wheat flour on a weight by weight basis.

Crumb firmness values of all breads containing 5% or 15% added bran were significantly higher than that of the control. There were significant differences in crumb firmness among all the samples supplemented with 5% or 15% wheat bran. Crumb firmness values of all breads containing 15% added bran were significantly higher than of the control and the breads baked from formulations containing 5% bran. The effect of high-fiber ingredients on crumb firmness was also studied by Hamid and Luan (2000). They reported that crumb firmness of bread baked from hard wheat flour was significantly lower than that of hard wheat flour containing 5% FIBREX, a commercial fiber.

Table 6.3. Quality Properties of Bread*

Sample	Bread Weight (g)	Firmness (N)	Bread Volume (cm^3)
HWF	127.5 ± 4.7a	2.92 ± 0.01a	840.0 ± 14.1f
HWF-5NWB	137.9 ± 0.7b	4.19 ± 0.16d	697.5 ± 3.5e
HWF-5WB	139.4 ± 2.2c	3.70 ± 0.08c	695.0 ± 7.1e
HWF-5ENWB	139.5 ± 2.0c	3.12 ± 0.10b	692.0 ± 3.5e
HWF-5EWB	140.7 ± 2.0d	4.49 ± 0.08e	695.0 ± 7.1e
HWF-15NWB	144.7 ± 1.6e	4.62 ± 0.19f	645.0 ± 7.1d
HWF-15WB	151.7 ± 2.4f	5.97 ± 0.08g	630.0 ± 7.1c
HWF-15ENWB	147.9 ± 1.5g	6.57 ± 0.25h	617.5 ± 3.5b
HWF-15EWB	151.9 ± 2.0h	9.36 ± 0.11i	610.0 ± 14.1a

*Values followed by the same letter in the same column are not significantly different ($p<0.05$); for abbreviations, refer to Table 6.1.
Values are means of two determinations ± standard deviation.

6.4.4. Cookie Quality Properties

Diameter, height, and hardness of cookies baked from soft wheat flour substituted with 5% or 15% of non-washed, washed, extruded non-washed and extruded washed bran samples are listed in Table 6.4. Cookie weights were not significantly affected by adding bran samples to cookie formulations. Cookie diameter decreased whereas cookie height increased with increasing bran substitution. Cookie hardness was higher for cookies made from soft wheat flour containing 5% or 15% bran samples compared to the cookies made from control flour. The results of the present study are in agreement with those obtained by Uysal et al (2007), Gujral et al (2003), and Tangkanakul et al (1995). They reported that increasing the concentration of apple fiber in the cookie formulation results in decreased cookie spread. The diameters of cookies with 4, 8, or 12 % (w/w) added apple fiber were significantly smaller than the control (Chen et al 1988). Chen et al (1988) reported that addition of 12% wheat bran decreased cookie spread. James et al (1989) found that adding rice bran to cookies reduced their crispness and their diameters.

The effect of wheat bran to decrease cookie spread may be related to its water binding properties (Vratania and Zabik 1978; Gorczyca and Zabik 1979; Jeltema et al 1983; Sievert et al 1990; Artz et al 1990; Chen et al 1988). In the present study, the reduced diameters of cookies baked from soft wheat flour containing wheat bran may be caused by the higher water binding capacity of wheat bran. It is possible that during baking, binding of water by wheat bran leaves less water in the system, water that would otherwise be used to dissolve sucrose and increase viscosity, which is responsible for cookie spread.

Table 6.4. Effect of Bran Substitution on Quality Properties of Cookies*

Sample	Weight (g)	Diameter (cm)	Height (cm)	Hardness (N)
SWF	20.82 ± 0.03ab	8.09 ± 0.02f	0.91 ± 0.01ab	27.6 ± 0.8a
SWF-5NWB	21.56 ± 0.04ab	7.68 ± 0.05d	0.93 ± 0.01bc	32.3 ± 1.3abc
SWF-5WB	20.18 ± 0.03a	7.46 ± 0.02c	1.01 ± 0.01d	29.0 ± 1.2ab
SWF-5ENWB	20.85 ± 0.03ab	7.74 ± 0.02e	0.94 ± 0.02c	32.3 ± 1.3abc
SWF-5EWB	21.76 ± 0.02b	7.29 ± 0.06b	1.01 ± 0.02d	30.4 ± 0.8abc
SWF-15NWB	21.65 ± 0.04b	7.76 ± 0.02e	0.89 ± 0.01a	29.5 ± 1.6ab
SWF-15WB	21.36 ± 0.02ab	7.14 ± 0.05a	1.03 ± 0.02d	32.4 ± 0.0abc
SWF-15ENWB	21.47 ± 0.02ab	7.68 ± 0.03d	0.94 ± 0.02c	35.8 ± 1.9c
SWF-15EWB	21.74 ± 0.02b	7.11 ± 0.03a	1.08 ± 0.02e	34.0 ± 1.30bc

*Values followed by the same letter in the same column are not significantly different ($p<0.05$);
SWF: Soft
Wheat Flour; for all other abbreviations refer to Table 6.1.
Values are means of two determinations ± standard deviation.

6.4.5. Effect of Baking on the Insoluble and Soluble Dietary Fibers in Bread

The contents of insoluble and soluble dietary fiber presented in Figures 6.1 and 6.2 were calculated based on equal weight of flour on a dry weight basis. According to the results presented in Figure 6.1, the contents of insoluble dietary fiber in crumb and crust of bread made from hard wheat flour were not significantly different from each other but significantly higher than insoluble dietary fiber in hard wheat flour (HWF). The contents of insoluble dietary fiber in crumb and crust of bread made from wheat flour substituted with 15% wheat bran (HWF-15B) were significantly higher than the control unbaked flour substituted with 15% bran (HWF-15). On the other hand, baking significantly increased the contents of soluble dietary fiber with higher increases reported in crust than in crumb (Fig. 6.2). For the hard wheat flour substituted with 15% wheat bran, baking significantly increased soluble dietary fiber in the bread crust whereas the soluble dietary fiber was not significantly different from that in the control (HWF-15, Fig. 6.2).

Vitaglione et al 2008 reported that starch gelatinization and retrogradation together with the formation of protein-polysaccharide complexes through Maillard reactions could be responsible for the increases in insoluble dietary fiber in processed foods, as long as long as processing conditions are favorable for chemical reactions to take place. Wang et al (1993) reported that high processing temperature could disrupt covalent and non-covalent bonds in the carbohydrate-protein complexes resulting in smaller fragments with increased solubility. The results from this study reveal that dietary fiber distribution varies within a loaf of bread with the crust containing more soluble fiber than the crumb. Substituting a certain portion of wheat flour with wheat bran is one way to increase insoluble dietary fiber in food products. However, addition of wheat bran to hard wheat flour is not the best way to increase soluble dietary fiber in bread.

This may be due to the limited amount of soluble dietary fiber present in wheat bran. Overall, substituting wheat flour with wheat bran increases total dietary fiber in bread.

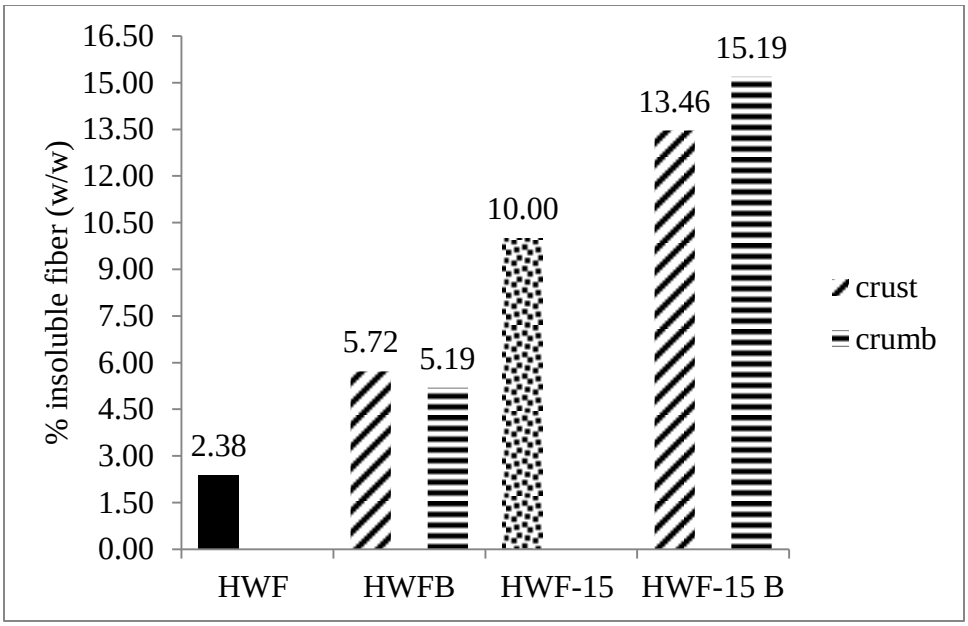

Figure 6.6. Insoluble dietary fiber in flour samples and its quantity and distribution in subsequent baked bread.

HWF: hard wheat flour; HWFB: bread baked from hard wheat flour; HWF-15: hard wheat flour substituted with 15% bran (w/w); HWF-15B: bread baked from hard wheat flour substituted with 15% bran (w/w).

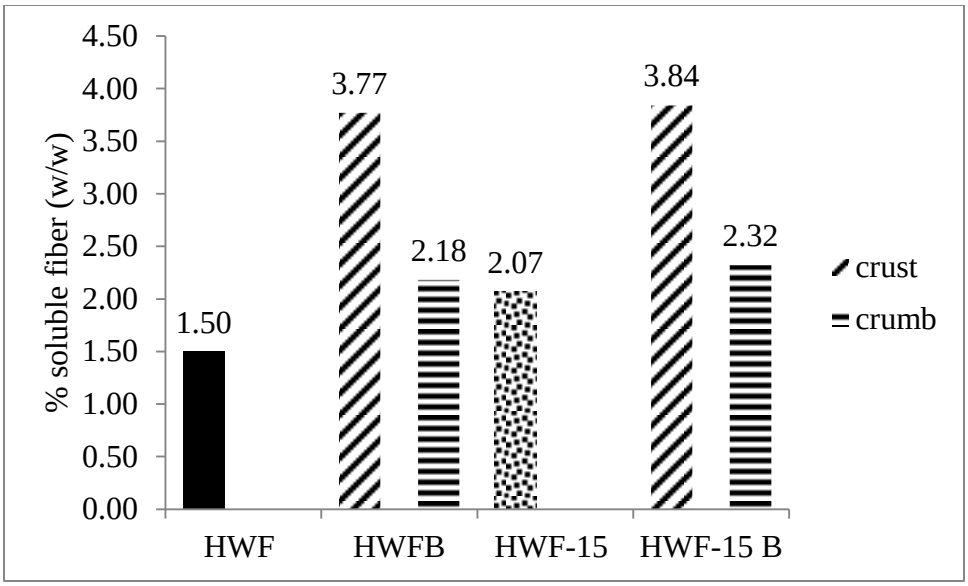

Figure 6.7. Insoluble dietary fiber in flour samples and its quantity and distribution in subsequent baked bread.

For abbreviations, refer to Fig. 6.1.

6.5. CONCLUSIONS

The results from the present study show that addition of wheat bran to bread and cookie formulations affect the physicochemical properties of the doughs and endproducts. Addition of wheat bran increased Farinograph water absorption of hard wheat flour. Also, addition of bran to hard and soft wheat flours decreased the pasting viscosities, as analyzed by the Rapid Visco Analyzer. Bread and cookie formulations containing bran resulted in lower loaf volume and cookie spread, respectively. Breads containing 15% bran had lower volumes compared to those containing 5% bran. Increasing the concentration of wheat bran to 15% decreased loaf volume and increased bread firmness. Increasing wheat bran in the cookie formula did not significantly affect cookie hardness. The contents of insoluble and soluble dietary fiber were higher in bread than in control flours.

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CHAPTER 7

GENERAL CONCLUSIONS

Wheat bran, a by-product of dry milling of wheat, contains substantial amounts of minerals and vitamins and is rich in dietary fiber than any other part of a wheat kernel. It is technically impossible to obtain bran free of endosperm during the process of roller milling of wheat as there is always residual starchy endosperm still adherent to the bran. Previous studies on the effect of processing, such as particle size distribution and extrusion processing conditions on the composition, physicochemical, and physiological properties of wheat bran, have been conducted using non-washed wheat bran. The neglect by previous investigators of the residual starchy endosperm adherent to wheat bran motivated the present author to investigate the effect of this adherent starchy endosperm on dietary fiber composition, physicochemical properties, and functionality of wheat bran. Therefore, the specific objectives of the present study were: (1) to develop a method that removes most of the residual starchy endosperm still adherent to the milled wheat bran, (2) to investigate the effects of particle size of non-washed and washed wheat bran coupled with extrusion processing variables on insoluble and soluble dietary fiber contents of the bran, (3) to investigate the effects of extrusion cooking conditions on the physicochemical properties of extruded non-washed and washed wheat bran samples, and (4) to evaluate the effects of treated wheat bran on the baking properties of bread and cookie products.

A washing method was developed to remove as much as possible of the residual starchy endosperm still adherent to wheat bran. Washed bran was then ground to pass through 1000 μm (WB1000) and 425 μm (WB425) screens. Non-washed bran ground to pass through 1000 μm (NWB1000) and 425 μm (NWB425) screens was used as controls. The washing method developed in the present study decreased total starch content in washed bran samples by over 70%. Transition enthalpies of washed bran samples, as measured by Differential Scanning Calorimetry, were much smaller compared to those obtained from non-washed bran samples,

which indicated that the washing method removed significant amounts of residual starchy endosperm. The insoluble dietary fiber contents in WB1000 and WB425 were significantly higher than those in NWB1000 and NWB425. On the other hand, soluble dietary fiber contents were significantly lower in washed bran samples than in non-washed bran samples. Total dietary fiber in NWB1000 was significantly higher than that it NWB425. However, the insoluble, soluble, and total dietary fibers in WB1000 and WB425 were not significantly different from each other. The water binding capacity was significantly higher in NWB1000 than in NWB425. On the other hand, the water binding capacities of the washed wheat bran samples were not significantly affected by particle size of the bran. Based on the findings of the present study, it is evident that the presence of residual starchy endosperm on milled wheat bran can easily interfere with analyses that are used to determine a bran sample's composition and physicochemical properties.

An extrusion study was conducted to investigate the effects of extrusion cooking on the contents of insoluble and soluble dietary fibers in non-washed and washed bran samples. Most of the extrusion cooking conditions decreased insoluble dietary fiber and increased soluble dietary fiber contents in NWB425 and NWB1000. On the other hand, extrusion of WB425 did not have a defined trend with regard to insoluble and soluble dietary fiber contents. Insoluble dietary fiber contents in extruded WB1000 samples were significantly lower than in the non-extruded WB1000, but soluble dietary fiber contents either increased or decreased during extrusion. It is not clear if the fluctuation in soluble dietary fiber contents in washed bran samples was due to the effects of extrusion conditions or if the level of dietary fiber content present was below the sensitivity level of the assay used.

The present study also examined the effects of extrusion cooking conditions on the physicochemical properties, such as water binding capacity, molecular weight, binding of bile

acids, and thermal degradation, of extruded non-washed and extruded washed wheat bran samples. The results of the present study indicate that the water binding capacity values of extruded non-washed bran samples were significantly higher than those of non-extruded NWB1000 and NWB425. Extrusion conditions increased the values of water binding capacity in non-washed wheat bran samples. However, certain extrusion conditions increased or decreased the water binding capacities of washed bran samples. Washing and extrusion cooking decreased molecular weights of soluble dietary fiber in both non-washed and washed bran samples. Thermal decomposition properties of wheat bran samples were not affected by extrusion. The onset decomposition temperature and peak decomposition temperature of washed bran were lower than those of non-washed bran. On the other hand, decomposition enthalpies of non-extruded and extruded washed bran were significantly higher than those of non-extruded and extruded non-washed bran. Extrusion increased i*n vitro* binding of bile acids in extruded NWB1000 and WB1000.

The results of baking studies indicated that addition of non-washed, washed, extruded non-washed, and extruded washed wheat bran samples affected the quality properties of baked bread and cookies. Substituting hard wheat flour with 5% or 15% of wheat bran sample increased its Farinograph water absorption. Wheat bran was found to reduce the pasting viscosities of hard and soft wheat flour samples. Breads containing wheat bran had decreased bread volume compared to the wheat flour-only control. Cookie formulations containing bran produced cookies with reduced spread. Quality properties of bread and cookies deteriorated with an increase in bran substitution.

Overall, the findings obtained from the present study indicate that the residual starchy endosperm still adherent to milled wheat bran affects analyses of the insoluble and soluble dietary

fiber profile as well as other physicochemical properties of the bran sample. The lower values of soluble dietary fiber in washed bran indicate that residual starchy endosperm adherent to milled wheat bran is high in soluble dietary fiber. Expanding upon these findings, it may be possible for the soluble dietary fiber content in milled wheat bran to be increased by altering the mill settings as well as by treatment of wheat grain before milling to obtain wheat bran with substantial amounts of residual endosperm. In addition, this scientific information could be used by wheat breeders to develop varieties that contain residual endosperm that cannot easily be peeled off during the milling process. This would increase the amount of soluble dietary fiber content in high bran-containing cereals, such as "All Bran" cereal. On the other hand, the washing method developed in this study could be used by the food industry to make pure wheat bran, as ingredients for novel products.

The findings of this study indicate that the bran's residual starchy endosperm affects the dietary fiber profile and physicochemical properties, such as water binding capacity of milled wheat bran. Wheat bran that was treated to remove most of the residual starchy endosperm (washed wheat bran) contained substantial amounts of insoluble dietary fiber and had a higher water binding capacity as compared to non-washed wheat bran. Insoluble dietary fiber content and water binding capacity greatly affect physiological properties of wheat bran. Therefore, washed wheat bran could be used as a functional ingredient in products intended to improve intestinal regulation.

Extrusion processing conditions decreased the contents of insoluble dietary fiber and increased the contents of soluble dietary fiber in all extrudates made from non-washed bran. On the other hand, extrusion decreased insoluble dietary fiber but did not increase soluble dietary fiber in all extrudates made from washed wheat bran. This indicates that the residual starchy

endosperm still adherent to milled wheat bran undergoes certain chemical modifications during extrusion cooking to form new components that have properties similar to those of soluble dietary fiber. This phenomenon contradicts previous investigators who suggested that extrusion shear results in breakage of the glycosidic bonds of insoluble dietary fiber, thereby increasing soluble dietary fiber in non-washed bran.

The fact that binding of bile acids was not affected by the amount of soluble dietary fiber present in the sample does not mean that soluble dietary fiber is unable to decrease serum cholesterol. There may be other mechanisms by which soluble dietary fiber decreases cholesterol in the blood. Unlike cholestyramine, which decreases cholesterol in the blood through ionic binding of bile acids in the intestine, the mechanism by which soluble dietary fiber removes cholesterol from the body may be different. Because soluble dietary fiber binds water to form a viscous gel, it is possible that bile acids are physically trapped by the gel and eventually end up in the feces.

The ability of wheat bran to provide physiological benefits such as fecal bulking is affected by their its binding capacity. Previous studies have indicated that the fecal bulking capacity of large particle size bran is higher than that of small particle size bran. The explanation for this phenomenon is that the water binding capacity of large particle size bran is higher than that of small particle size. The results of the present study showed that large particle size bran is higher in insoluble dietary fiber than is small particle size bran. Therefore, it can be speculated that the increased fecal bulking by large particle size wheat bran is due to its higher insoluble dietary fiber content. In addition, the results of the present study indicated that particle size of washed wheat bran had no effect on water binding capacity and the insoluble dietary fiber content of that bran sample. If fecal bulking depends on the water binding capacity and insoluble dietary

fiber content, it can be suggested that fecal bulking is not affected by particle size of washed wheat bran. However, it is important to note that the smaller particle size bran increases surface area available for bacterial fermentation in the large intestine. Increased fermentation impairs the ability of fiber to increase fecal bulking.

CHAPTER 8

FUTURE RECOMMENDATIONS

1. Injecting water into the extruder barrel during extrusion does not provide enough time for the water to penetrate the bran. It is recommended that future studies investigate how extrusion of wheat bran that has been pre-soaked may affect its physicochemical properties. Extruding soaked wheat bran may increase heat transfer and facilitate cooking during extrusion, and change the soluble and insoluble fiber distribution.
2. Generally, extrusion conditions changed dietary fiber distribution in non-washed and washed bran samples. It is recommended that further studies examine the effects of extrusion conditions on the composition of dietary fiber monosaccharides, such as rhamnose, arabinose, xylose, mannose, galactose, and glucose.
3. Extrusion processing increased or decreased soluble dietary fiber in washed bran samples. It is not clear if these were actual changes due to extrusion conditions, or if the protocol used to determine soluble dietary fiber was not sensitive enough to accurately measure low levels of soluble dietary fiber. More studies are recommended to examine this phenomenon.
4. Addition of wheat bran to bread formulation decreased loaf volume. Future studies should examine effects of bran substitution together with addition of dough enhancers, such as vital gluten, on quality properties of bread.
5. Nutritional studies have indicated that large particle size non-washed wheat bran increases stool volume to a greater degree than small particle size non-washed wheat bran. In these studies, water binding capacity of bran has been identified as an important factor that affects physiological functions of fibers. The results of the present study indicate that the water binding capacities of small and large particle size washed brans were not

significantly different from each other. Therefore, it is recommended that further studies investigate the effect of particle size of washed wheat bran on fecal bulking.