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# Effects of Bran Treatment on Rheology and Sensory Quality of Whole Wheat Flat Bread

Jigyasha Mishra
*South Dakota State University*

# EFFECTS OF BRAN TREATMENT ON RHEOLOGY AND SENSORY QUALITY OF WHOLE WHEAT FLAT BREAD

BY

JIGYASHA MISHRA

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EFFECTS OF BRAN TREATMENT ON RHEOLOGY AND SENSORY QUALITY OF WHOLE WHEAT FLAT BREAD

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Biological Sciences with a specialization in Food Science and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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| Padmanaban Krishnan, Ph.D.<br>Major Professor and Thesis Advisor | Date |
| Vikram Mistry, Ph.D.<br>Head, Department of Dairy Science | Date |
| Dean, Graduate School | Date |

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# ABBREVIATIONS

| | |
|---|---|
| AACC | American Association of Cereal Chemists |
| AOAC | Association of Official Analytical Chemists |
| BT | Farinograph breakdown time |
| BU | Brabender unit |
| CHD | Coronary heart disease |
| CODEX | Codex Alimentarius Commission |
| CVD | Cardiovascular diseases |
| db | Dry basis |
| DDT | Dough development time |
| DF | Dietary fiber |
| DS | Dough stability |
| $E_{diff}$ | Difference between $E_{max}$ and $E_{Rmax}$ |
| $E_i$ | Gradient of force distance curve during first second of test |
| $E_{max}$ | Maximum dough extensibility |
| $E_{Rmax}$ | Extensibility of dough and $R_{max}$ |
| FAO | Food and Agriculture Organization |
| GI | Gluten Index |
| HRSW | Hard red spring wheat |
| HRWW | Hard red winter wheat |
| LDL | Low density lipoprotein |
| MTI | Mixing tolerance index |
| MN | Minnesota |

| | |
|---|---|
| Nm | Newton meter |
| TDF | Total dietary fiber |
| U.S. | United States of America |
| USDA | United States Department of Agriculture |
| SD | South Dakota |
| SRSW | Soft red spring wheat |
| SRWW | Soft red winter wheat |
| WA | Water absorption |
| WASDE | World Agricultural Supply and Demand Estimates |
| wb | Wet basis |
| WHO | World Health Organization |
| WWF | Whole wheat flour |

# ABSTRACT

# EFFECTS OF BRAN TREATMENT ON RHEOLOGY AND SENSORY QUALITY OF WHOLE WHEAT FLAT BREAD

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The purpose of this research was to study the differences in the quality of 100% whole wheat flour (WWF) when the bran fraction was subjected to mild solvent treatment before reconstitution with the endosperm. The bran treatments included water washing and washing with ethanol solutions (50% and 100%). WWF prepared with untreated bran served as the control. Effects of these treatments on particle size, chemical composition, color, dough rheology, product formulation, and sensory characteristics of 100% whole wheat flour were studied. Grains of five HRSW cultivars (Advance, Prevail, Select, Brick and Forefront) were milled using a Quadrumat Senior mill.

Gluten analysis of patent white flour obtained from five HRSW showed Brick and Advance to have superior gluten quality for bread baking. Washing the bran with 50% ethanol significantly lowered redness (a*) while washing the bran with 100% ethanol significantly lowered yellowness (b*). Brick cultivar was found to be superior in Farinograph data in relation to dough development time (DDT), dough stability (DS), breakdown time (BT), and mixing tolerance index (MTI). Of the four bran treatments used, washing with 50% resulted in significantly higher DDT, BT and lower MTI, in contrast to the control. Washing with 100% ethanol resulted in significantly higher DS and lower MTI compared to the control. Cultivars Brick and Prevail had significantly

high $R_{max}/E_i$ and cultivars Advance and Forefront had the lowest $R_{max}/E_i$. Flour with 50% ethanol-treated bran had the highest $R_{max}/E_{Rmax}$ and control (WWF) had the lowest. Control (WWF) had significantly high $E_{max}$ while flour with ethanol treated bran (both at 50% and 100% strength) had significantly low $E_{max}$. Brick and Prevail were superior varieties in terms of dough extensibility. Results from the one dimensional extensibility test indicated that tortillas made from the flour incorporated with 50% and 100% ethanol-treated bran were good in relation to stretchability, while the control (WWF) was found to be desirable in relation to elasticity. Treatments of bran were not discerned in tortilla colors and sensory attributes.

# CHAPTER 1 INTRODUCTION

## 1.1. Introduction

The study of whole grain has intensified in recent years. It has been more evident from those studies that the whole-grain cereal products help to protect consumers from the development of chronic metabolic diseases (Adil, 2012; Anthony Fardet, 2010). This prevention on the development of the metabolic diseases is the result of the synergistic action of the compounds contained in the bran and germ fractions of whole-grain cereals (A. Fardet, 2010). Obesity, metabolic syndrome, type 2 diabetes, CVD, and cancer are some of the major metabolic diseases that have threatened the public health (Soumya, 2011; Uslu, et al., 2013). These metabolic diseases are mostly due to our day to day lifestyle and food consumption, where the major meals consist of an unbalanced energy-rich diet which lacks fiber and protective bioactive compounds such as micronutrients and phytochemicals. The root cause of metabolic diseases is an unbalanced dietary habit amongst consumers. There is now a need for healthy whole grain foods that mitigate risk factors and diseases.

Adults in the United States consume less than the recommended value of dietary fiber. Therefore, the production and consumption of high-fiber foods should become more predominant in the United States. The two conventional dietary fiber sources are wheat and oat bran (H. Chen, et al., 1988). The American Association of Cereal Chemists (AACC) defined dietary fiber (DF) 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; that includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Wheat bran is endowed with health beneficial bioactive compounds, such as tocopherols, phenolic compounds, and other organic acids. Bran, thus obtained from the debranning process, can be used for the production of functional foods and nutraceuticals (Y. Chen, et al., 2013).

As a proportion by weight, the wheat kernel is composed of an outer bran layer (14–16% of the grain), the germ or embryo (2–3%), and the central endosperm (mainly starch: 81–84%) (Stevenson, et al., 2012). The germ and bran are removed from endosperm during milling because of their unfavorable baking characteristics and susceptibility to oxidation. These fractions are used as livestock feed. Wheat germ is used for oil production. These co-products contain healthy food constituents (Kumar & Krishna, 2013). However, they are underutilized for consumption and not used in food production. Wheat bran represents a good source of fiber, minerals, vitamins and phytochemicals. Dietary fiber (DF) plays an important role in the human health and exhibits many functional properties of food. DF also reduces the risk of serious human diseases such as colorectal cancer, cardiovascular disease, and diabetes (Reyes-Pérez, et al., 2013). Several papers have been published on the effective use of wheat bran (Anderson, et al., 2009; Balandrán-Quintana, et al., 2015a; Dykes & Rooney, 2007; A. Fardet, 2010; Sobota, et al., 2015). The main purpose of this paper is to highlight effects of mild treatments on bran and study the quality of whole wheat made with the bran.

Generally, lipids from biological materials have always been extracted by the use of conventional organic solvents. The most widely used solvents are petroleum ether, ethanol and hexane. Petroleum ether is most widely used because of its low boiling point ($20^0$C to $75^0$C) and low cost. This paper will be focused on the use of alcohol for bran treatment so that the treated bran can be incorporated back into the patent flour to yield whole wheat flour with better characteristics than the regular whole wheat flour in terms of rheological properties and dough extensibility. Reincorporation of bran into patent flour, as part of whole grain food, will improve nutrition and health, as bran contains health promoting bioactive constituents. Undesirable taste from bran constituents may also be selectively removed.

This study evaluated ethanol-treated bran from SD wheat varieties. Consumers like the taste of the food products such as Asian noodles and bread products when these foods are not bitter. Thus, treated-wheat bran can serve as a novel ingredient in most food products such as bread, pizza crust and cookies. So this method of bran treatment is likely to improve the taste of bran so that it could be incorporated into any food product in order to increase dietary fiber content. This incorporation can also bring about some rheological changes and may improve baking properties. Farinograph and dough extensibility tests were done to measure the resistance of dough to mixing.

1.2. Problem statement

Color is a key quality parameter that often affects consumer acceptability of food products like bread. Since the wheat bran is dark in color, the color of whole-wheat products are also usually dark.  In order to make the whole-wheat products more visually attractive to consumers and to retain the functionality of whole wheat, there is a need for techniques that will not affect the product quality, health attributes or sensory characteristics.

Production of whole wheat flour by the addition of treated bran can bring improvements in dough extensibility and dough rheological properties in terms of water absorption, dough stability, dough development time, and mixing tolerance index (MTI). These tests are used as rheological parameters in the industry to estimate the optimal amount of water to be added to make a dough, to evaluate the effects of ingredient on mixing properties and to check the overall flour uniformity. Desirable dough quality includes higher water absorption and dough stability with lower tolerance index and lower peak development time.

The main purpose of this research was to perform bran treatments using mild washing with water and ethanol solutions to enhance the color of whole wheat flour so as to increase the consumption of fiber-rich whole wheat foods among consumers. Products like bagel, regular bread, flat bread, pizza crust, and cookies made from such flour with treated bran will have improved color and maintained the same health benefits as provided by 100% whole wheat flour. DF’s significant health benefit includes decreased risk of cardiovascular disease. Grooms, et al. (2013) examined trends in dietary fiber intake among diverse US adults from the year 1999-2010 and investigated the association between dietary fiber and cardiovascular disease and obesity. The results showed that the intake of dietary-fiber among US adults was below the recommended value by the Institute of Medicine (IOM). Mean dietary-fiber intake was only 15.7-17 g per day whereas the recommended value is 38 g/day for males and 25 g/day for females. This study also confirmed the association of dietary fiber with lower level of inflammation and metabolic syndrome or obesity, though a difference in levels among various racial and ethnic group was observed. Thus there is a need of developing the strategy and policies to increase the consumption of dietary fiber.

The purpose of this study was to determine the effective treatment of wheat bran using 50% ethanol, 100% ethanol, and water as a mild solvent. The working conditions of the process (solvent concentration, soaking time etc.) and the pretreatment of the raw material (i.e., tempering and milling) were optimized. Then a comparison was made between the relative qualities of the flour incorporated with the bran after ethanolic extraction.

### 1.3. Research Objectives and Hypothesis

#### 1.3.1. Research Objectives

The main objectives of this study were:

1. Study the effect of different concentrations of ethanol treatment on wheat bran,
2. Study the rheological properties and extensibility of whole wheat dough resulting from the incorporation of treated bran (15%) back to patent flour and to compare it with control which was 100% whole wheat flour (with untreated bran),
3. Compare different varieties of Hard Red Spring Wheat for their contribution to 100% whole wheat flour,
4. Study the particle size distribution of whole wheat flours,
5. Study the acceptability of products (tortilla) fortified with treated bran by sensory panels, and
6. Compare the uniaxial extensibility of wheat flour tortillas using Tortilla/Pastry Burst Rig (HDP/TPB)

#### 1.3.2 Hypothesis

1. H0: Treated bran will not be lighter in color due to the loss of pigments.

   H1: Treated bran will be lighter in color due to the loss of pigments.

2. H0: An improved dough rheological properties and dough extensibility will not be achieved from the incorporation of treated bran back into the patent flour.

   H1: An improved dough rheological properties and dough extensibility will be achieved from the incorporation of treated bran back into the patent flour.

3. H0: Significant effect on one dimensional extensibility of tortilla will not be observed.

   H1: Significant effect on one dimensional extensibility will be observed.

4. H0: Tortilla fortified with treated bran will not be acceptable to a trained sensory panel.

   H1: Tortilla fortified with treated bran will be acceptable to a trained sensory panel.

# CHAPTER 2. LITERATURE REVIEW

## 2.1. Background of wheat

Wheat, maize and rice are the most widely used cereals and is followed by oats, rye, barley, triticale, millet, and sorghum. Wheat was one of the first domesticated food crops and has been the staple food for the major civilizations of Europe, West Asia and North Africa for 8000 years (Curtis, et al., 2002). Wheat is grown more than any other food crops. The following definition of whole grain was approved by American Association of Cereal Chemist (AACC) in 1999: "Whole grains shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components; the starchy endosperm, germ, and bran—are present in the same relative proportions as they exist in the intact caryopsis."  World agricultural supply and demand (WASDE) states that approximately 652.18 million ton of wheat was consumed only in 2010. Bran is created as a by-product in milling industries and one million tons of wheat can produce up to 0.25 million tons of wheat bran (Javed, et al., 2012).

As shown in the figure 1, China is the largest producer of wheat, followed closely by India, the United States and Russia Federation, according to FAO statistics, 2011. On the basis of season during which the crop is grown, wheat is classified into spring or winter wheat. Commercially grown wheats are classified as hard wheat and soft wheat. Hard wheat is usually darker in color and has no white starch, while soft wheat is lighter in color and has white starch. Hard red winter wheat (HRWW) and hard red spring wheat (HRSW) are mainly used in bread making, while soft red winter wheat (SRWW) and soft red spring wheat (SRSW) are primarily used for making pastries, cookies, pies, and cakes.

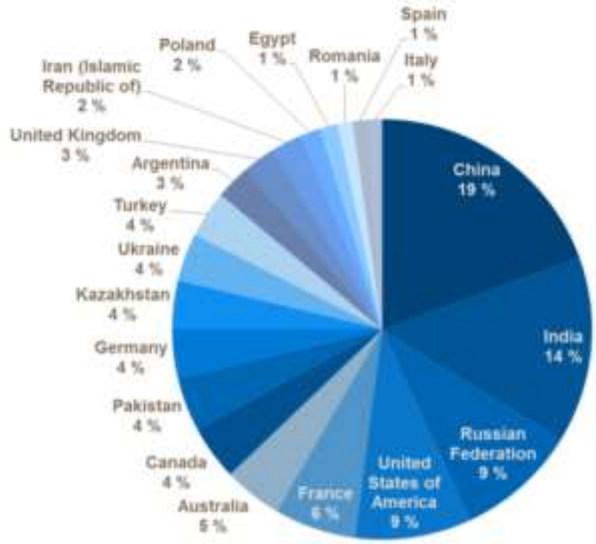


Figure 1.World wheat production by country in the year (2011).

Source: FAOSTATS 2011.

## 2.2. Structure of wheat grain

Wheat kernel is a caryopsis, which means, ovary wall is united with the seed coat. The dorsal side is smoothly rounded while the ventral side has the deep crease (Curtis, et al., 2002). As a proportion by weight, the wheat kernel is composed of an outer-bran layer (14–16% of the grain), the germ or embryo (2–3%), and the central endosperm (mainly starch: 81–84%)  (Stevenson, et al., 2012). Wheat bran contains approximately, 12% water, 13-18% protein, 3.5% fat, and 56% carbohydrates (Prückler, et al., 2014).

Bran is represented by three discrete layers. The three layers are aleurone and hyaline layer, inner and outer pericarp and testa or seed coat. The seed coat is the outermost layer of the true seed. Wheat kernel is completely surrounded by a coat formed by the seed coat and the pigment strand, however, the latter are different tissues. Red-brown pigmentation is found in both the seed coat and pigment strand in red wheat while it is absent from the seed coat and pigment layer in case of white wheat.  The pericarp is the ripened ovary wall that is dead at harvest ripeness. It is composed of an outer epidermis, hypodermis, parenchyma, intermediate cells, cross cells, and tube cells. The pericarp is rich in insoluble dietary fiber (like cellulose, cuticle material and complex xylans), lignins, ferulic acid, and other bioactive compounds. The aleurone layer is rich in proteins, minerals, and mostly Vitamin B. Dietary fiber and other bioactive compounds are also concentrated mostly in the aleurone layer.  Testa is the reserved source of almost all alkylresorcinols (a phenolic lipid).

The wheat germ or embryo is located on the lower dorsal side of the grain. It is reported to be an excellent source of B-group vitamins, unsaturated fatty acids, minerals, dietary fiber, calories, and proteins (Ge, et al., 2000). In general, the protein content of wheat fraction is greatest in germ followed by middlings, bran, and white flour (Pomeranz, 1988b). The germ is also the richest source of tocopherols. Thus wheat germ is best for the enrichment of processed foods. Because of the presence of unsaturated fatty acids and oxidative and hydrolytic enzymes, which are responsible for rancidity, the wheat germ has short shelf life (Sudha, et al., 2007).

The endosperm is the largest constituent of wheat grain. It is composed of aleurone cells and starchy endosperm. Aleurone cells in wheat are only one cell layer thick at maturity. Aleurone cells form the outermost layer of endosperm tissue, surround the grain over the starchy endosperm and part of the embryo. The aleurone layer is the source of vitamins, minerals, proteins, and lipids. It is also excellent source of natural folate, a B-group vitamin. The starchy endosperm of mature seed is a good source of nutrition. It consists majority of carbohydrates in the form of simple starches. Endosperm constitute 55%-75% of total grain weight, with a storage protein content of 10-20% (Gillies, et al., 2012).

Table 1. Composition of wheat bran

| Components | g/100g Wheat bran |
|---|---|
| a. Non starch polysaccharide | |
| - Glucan | 10.5 |
| - Xylan | 18.3 |
| - Arabinan | 10.1 |
| - Galactan | 1.1 |
| b. Starch | 34 |
| c. Klason lignin | 5 |
| d. Crude Protein | 13.5 |
| Total | 92.5 |

Source: Javed, et al. (2012).

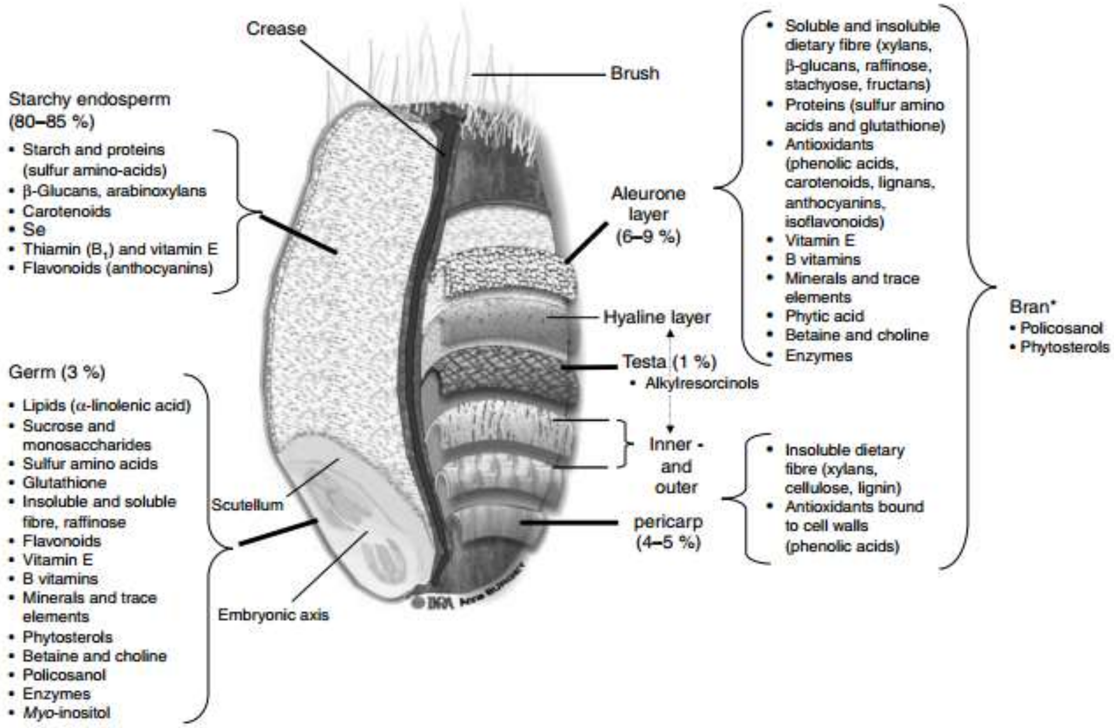


Figure 2. Wheat fraction; bran, germ and endosperm with their main bioactive compounds.

Source: (Anthony Fardet, 2010).

## 2.3. Health benefits of wheat bran

The bran fraction has many health benefits, as it is nutritionally rich in fiber, folic acid, vitamin B6, minerals, thiamine, and vitamin E. The bioavailability of these nutritionally rich compounds are dependent upon the food matrix and the processing conditions applied (Stevenson, et al., 2012). Wheat bran is beneficial for the treatment of chronic constipation, cardiovascular disease and diabetes (Müller-Lissner, 1988). This is all because of the presence of high level of dietary fiber and phytochemicals in bran (Balandrán-Quintana, et al., 2015b). Similarly, antioxidants (phenol) present in wheat bran have been shown to bind with apolipoprotein-B, thereby inhibiting LDL oxidation. Alkylresorcinol antioxidants present in wheat bran are also of great importance as they inhibit platelet by binding to fibrinogen, stimulate production of thromboxane and inhibits triglyceride formations. This suggests bran fractions to be a good source of phenolic compounds can be very useful for cardiovascular disease (Stevenson, et al., 2012). Similarly, insoluble dietary fiber can act as a bulking agent, increasing intestinal motility and wet fecal mass. That is why there is a growing interest among consumers towards whole cereal grain products as they protect against the development of chronic diseases (Anthony Fardet, 2010).

Though the protein content of wheat bran is not high, it is superior to the protein present in endosperm. Foods incorporated with wheat bran fiber resulted in decrease of glycemic index (Reyes-Pérez, et al., 2013). Various studies have suggested the health benefits of wheat bran. 42,850 male health professionals aged 40-75 years who were not suffering from cardiovascular disease, cancer and diabetes, completed a detailed self-administered food frequency questionnaire and medical history questionnaire. After 14 years of follow up there was only 1818 cases of CHD. Inverse association between intake of whole grain and bran and occurrence of CHD were seen (Jensen, et al., 2004).

The exact amount of fiber beneficial for health is not yet known. However, the recommended dose of dietary fiber per day is 25g, which is higher than people consume in most western countries. Since bread is used as staple food, more priority should be given towards the production and consumption of whole grain bread (Le Bleis, et al., 2015).

Table 2. Wheat bran components and their health benefits.

| Component | Beneficial action |
| --- | --- |
| Soluble Dietary fiber | Improves gut health, control glycemic index and reduces plasma cholesterol level |
| Dietary fiber | Prolongs bowel transition time and increases stool volume |
| Alkylresorcinol | Reduces human colon cancer cell growth |
| Ferulic acid | Antioxidant, anti-microbial, anti-inflammatory, anti-thrombosis, and anti-carcinogenic activities |
| Beta-glucan | Lower blood cholesterol and have vital role in insulin resistance, dyslipidemia, hypertension, and obesity |
| Arbinoxylan | Reduces postprandial glycemic response |
| Lignans | Protects against hormone related breast and prostate cancer |
| Sterols | Reduces total cholesterol |

Source: Prückler, et al. (2014).

Table 3. Average content (g/100 g food) of bioactive compounds in whole- grain wheat and wheat bran.

| Bioactive compound | Whole-grain wheat | Wheat bran |
|---|---|---|
| a-Linoleic acid (18:3n–3) | - | 0.16 |
| Sulphur compounds | 0.5 | 0.7 |
| Total free glutathione | 0.007 | 0.038 |
| Fiber (as AOAC) | 13.2 | 44.6 |
| Lignins | 1.9 | 5.6 |
| Oligosaccharides | 1.9 | 3.7 |
| Phytic acid | 0.9 | 4.2 |
| Minerals and trace elements | 1.12 | 3.39 |
| B vitamins | 0.0091 | 0.0303 |
| Vitamin E (tocopherols and tocotrienols) | 0.0047 | 0.0095 |
| Carotenoids | 0.00034 | 0.00072 |
| Polyphenols | 0.15 | 1.1 |
| Phenolic acids | 0.11 | 1.07 |
| Flavonoids | 0.037 | 0.028 |
| Lignans | 0.0004 | 0.005 |
| Alkylresorcinol | 0.07 | 0.27 |
| Phytosterols | 0.08 | 0.16 |

Source: Stevenson et al., 2012 (Modified).

## 2.4. Whole wheat products

Nowadays, wheat grain is gaining more popularity because of its numerous health benefits. Various fiber rich products are produced using wheat bran. The most popular products include whole wheat bread, enriched wheat bran cookies and wheat bran flakes. Some of the major wheat products are pasta and noodles, breakfast cereals, leavened bread, and flat bread like tortilla.

### 2.4.1. Pasta and noodles

Pasta and noodles are developed from unleavened dough. The main ingredients include salt, water and flour. Durum semolina is the ideal raw material for pasta (Hoseney, 1994) and for noodles it is white flour. Nowadays pasta and noodles made from whole wheat flour are found in market whose products are darker in color compared to semolina based pasta.

### 2.4.2. Breakfast cereals

Wheat flakes, shredded biscuit and puffed wheat cereals are some of the examples of breakfast cereal made from whole wheat flour. The whole wheats are tempered, steamed and bumped between smooth rolls and cooked in a pressure cooker with sugar, salt and malt flavor added. Wheat kernels are soft by this stage and contain 50% moisture. They are then dried, tempered and flaked in a flaking rolls.

One of the oldest ready to eat cereals made from whole wheat is shredded biscuits. Here, the whole wheat is boiled to increase the moisture content, tempered to equalize the moisture content and shredded in the shredding rolls of about 8 inch diameter and the length of a biscuit. They are grooved around the roll resulting in a wet, ground whole wheat paste. The dough strands are layered one upon another by 18-20 pairs of rolls which are later separated into biscuits by passing through blunt knives. The final step is the baking of the fragile biscuit at high temperature for about 15 minutes. Since this is made from 100% whole wheat, rancid odor may develop during storage so they are packed in breather type boxes with no inner or outer gas barriers (Hoseney, 1994). For the production of puffed cereals three techniques can be used:

- Use of heat at atmospheric pressure where water is vaporized before it could diffuse to the kernel surface and the internal vaporization then expands resulting into puff.
- Sudden transfer of a piece with superheated water at low pressure causing sudden vaporization of water
- Extrusion cooking which is continuous and uses both temperature and pressure for expansion of the product.

2.4.3. Whole wheat flat breads

Flat breads are an ancient type of bread amongst all breads and consumed all around the world. Only few people were familiar with flat breads in USA few years back but successful growth of restaurants all over the world with cultural roots in Greece, India, Mexico, Turkey, and various Arab countries and the growing interest in travelling have changed the people’s choice of food (Pomeranz, 1988b).

Table 4. Traditional flat breads made from whole wheat.

| Type of bread | Country or Region | Description |
|---|---|---|
| Korsan | Saudi Arabia | Flat, circular with a single thin layer made from whole wheat flour, water, and salt |
| Nepalese bread | Nepal, Northern India | Flat, triangular fried bread made of whole wheat flour, yeast (1%), water (30%), fresh milk (5%), sugar (1%), salt (0.5%), egg (2%), and small quantities of cinnamon, nutmeg, and cloves. |
| Paratha | India | Unleavened bread made from whole wheat flour, salt, water and shortening. |
| Raghif | Israel | Thick, flat bread made by peasants from whole wheat flour and water. |
| Moroccan whole wheat bread | Morocco | Round bread made from whole wheat flour, water, salt and yeast. After mixing all the ingredients, the dough is flattened, allowed to ferment for 3 hour, docked and baked in a relatively cool oven. |
| Tortilla | Mexico, Central America, United states | Unfermented, round flat bread prepared either from wheat or maize. |

Source: Pomeranz (1988a).

### 4.3.1. Tortilla

Tortilla, were originally homemade and consumed in Mexico for centuries. Tortillas are unfermented flat breads made mainly from three cereals namely, corn, wheat, and barley (Guo, et al., 2003). Comparatively, wheat tortillas are more popular in the US than other ethnic breads due to their versatility for various items (Alviola, et al., 2012; Guo, et al., 2003). Most wheat tortillas found in the market are made from all-purpose flour and are thus a limited source of fiber. Dietary fiber rich foods are whole grain but whole grains are consumed less by Americans. The consumption rate is only one serving instead of three servings of whole grain per day. This is because of the dark color and undesirable flavors of whole grain based products (Alviola, et al., 2012). Americans prefer wheat tortillas over corn tortillas in the ratio of 2:1 (Serna-Saldivar, et al., 2004). Whole wheat tortillas are a good source of dietary fiber (7-8%), 3% insoluble and 4.2% soluble fiber (Barros, Alviola, & Rooney, 2010). They are also rich in carbohydrates with high glycemic response after ingestion.

Flour tortillas are made by hot-press, die-cut or hand-stretch procedure (Anton, 2008; Barros, Alviola, et al., 2010a). Tortillas made from hot-pressing is soft and more flexible during storage which are perfect for burritos, soft tacos, fajitas, and wraps. The die cut method is more efficient with lower cost of the product but in terms of quality, hot-press is better. Comparatively, larger, thinner and stronger tortillas are made using hand-stretch method (Anton, 2008). Flour milled from hard red spring wheat (HRSW) are used for tortillas making (Serna-Saldivar, et al., 2004).


(a) (b)



(c) (d)


(e)

Figure 3. Various wheat bran products; (a) oat and wheat bran swirls, (b) whole wheat cookies (c) whole wheat flat bread (d) enriched wheat bran flakes and (e) whole wheat regular breads.

## 2.5. Wheat Milling

Wheat milling technology has evolved from the use of the mortar and pestle by primitive cultures nearly 10,000 years ago. This had led to the invention of millstones in Roman times. The invention of plan-sifter, purifier and horizontal roller mill revolutionized the milling technology during the late half of the 19$^{th}$ century (Sakhare & Inamdar, 2014). The most notable achievements in milling, including materials handling, further refinement and improvement of existing milling machinery, automation and computerization of the whole milling process, was achieved in the 20$^{th}$ century (Pomeranz, 1988b).

The wheat delivered to the mill requires much more expensive cleaning process to remove foreign materials such as stones, mud, ergot, metals, straw, and other seeds, which might affect the appearance or functionality of the milled product and the mill itself. Hence, wheat delivered in mill is first transferred from the elevator to the wheat-cleaning section. The wheat-cleaning section includes the use of a milling separator and aspirator, gravity separator or destoner and disc separator. A milling separator removes impurities that are marginally larger or smaller than wheat. A destoner separates wheat from other materials, such as small stones, glass and nonferrous metal, in terms of shape and size on the basis of difference in specific gravity. The destoner may not be efficient in removing slightly longer or shorter materials than wheat so either a disc separator or conveyor separator can remove these impurities.

After the cleaning process is completed, wheat is tempered or conditioned. Tempering is the controlled addition of moisture to wheat. The main two objectives of tempering is to toughen the outer surface of the wheat to avoid powdering during the milling process and to facilitate the physical separation of endosperm from bran. The tempering process also ensures that grinding produces the minimum level of damaged starch consistent with the hardness of the wheat and the end use of flour. Thus correct tempering of cleaned wheat is essential to ensure maximum milling efficiency and optimum performance in the final product. In tempering, a controlled amount of water is added to the measured quantity of wheat through spray nozzles enclosed in a screw conveyor. The tempered wheat is finally ready to be milled.

The purpose of wheat milling is to break open the grain and dissociate the starchy endosperm from other parts of wheat grain to get the white flour (Fistes, et al., 2013). The two main processes for flour milling are breaking and reduction. In the breaking process, the break rolls, which are corrugated are used to open up the grain. There are different sets of break rolls. The ground material leaving the first break roll, known as break chop, now passes to the sieving system. Here, sifting machines separate the mixture of particles according to size. The largest particles, referred as scalp, consist of the tough wheat bran and adhering endosperm. The scalp proceeds to second break roll where it is again ground on corrugated rolls and is sent to the second break sifter for further sifting. The endosperm particles larger than flour particles are called break middlings which is a mixture of pure endosperm, bran and endosperm attached to bran. The break middlings are sent to a grading system where they are separated according to their size. The middlings are separated into homogenous fractions in the purification system. More endosperm is scraped off as the materials are processed through different sets of break rolls until finally only the flat bran particles remain for final processing as a by-product

(Pomeranz, 1988b). Thus, the scalping sieve through which the endosperm is separated from bran coats becomes finer as the breaks proceed. In the reduction system, pairs of smooth rolls are used to reduce the larger particles of endosperm into flour particle size.

The effectiveness of milling process depends on the recovery of endosperm uncontaminated with bran. The amount of flour obtained after milling is the extraction rate. The extraction rate of wheat flour ranges from 73%-77% depending upon the milling process, wheat variety and cultivation conditions (Prückler, et al., 2014).

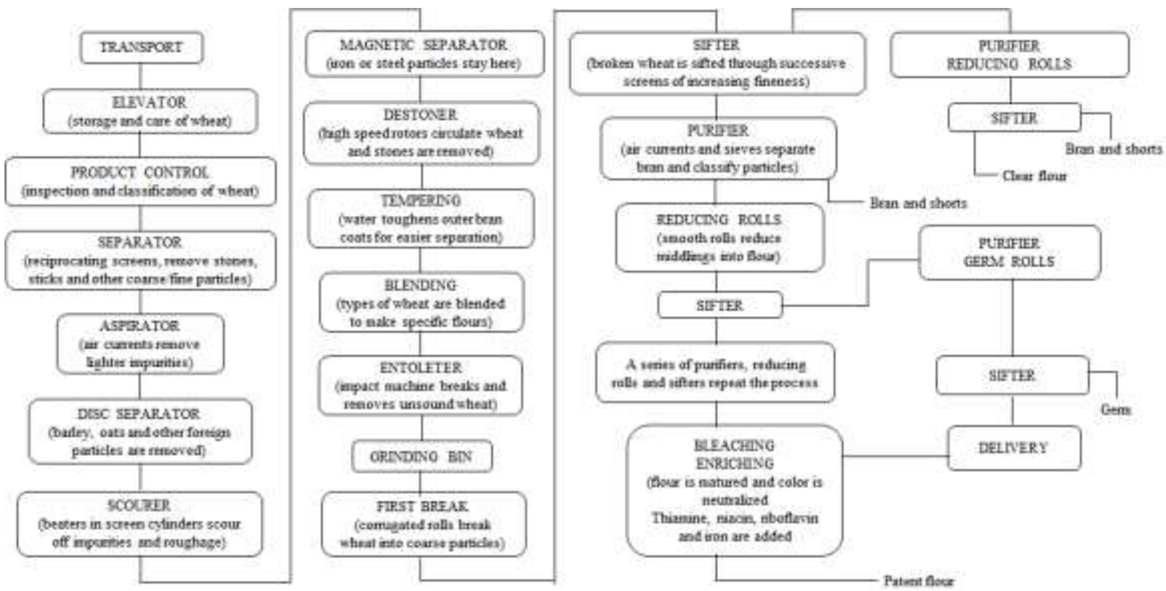


Figure 4. Flow diagram of the wheat milling process.

Source: Darly-Kindelspire (2013).

2.6. Wheat bran treatment

The addition of high levels of wheat fiber to foods may result in reduced bread loaf volume, poor texture, bitter flavor and a darker color. Various research has been conducted to modify wheat bran for food applications. Widely used modification methods include wet milling, enzymatic treatment, fermentation and chemical treatment. Chemical treatment involves the use of chemicals like citric acid, sodium hydroxide, hydrochloric acid, chloroform, methanol and ethanol. The extraction solvent's selection criteria depends on its physiochemical properties (selectivity, stability, and reactivity), toxicity and cost. Some organic solvents like hexane, chloroform, and methanol are restricted in food industry because of their toxicity. However, the presence of some solvents like ethanol, and acetone in small residual percentage is permissible for human consumption, according to good manufacturing practice (Meireles, 2008).

In the study by Rasco, et al. (1991), bran has been treated using a number of different chemicals (acid, base, ethanol) alone or in combination with enzymes (α-amylase or α-amylase/protease or α-amylase/calcium oxide). The purpose of the study was to enhance the dietary fiber content of wheat bran but the baking characteristics were negatively affected. In a recent study, ethanol has been used to remove the color pigments from corn dried distiller's grain with soluble (DDGS) to produce a high quality food ingredient (Sauders et al., 2013). The limiting factor responsible to bring the change in color were the number of extractions, ethanol concentration and time. Overall, treatment with ethanol resulted in reduction of oils and carotenoid contents in corn.

Our research was more focused on the use of 50% and 100% ethanol for wheat bran treatment as a method to improve the color and rheological properties of whole wheat dough. Use of ethanol and ethanol-water mixture for the recovery of phenolic compound and anthocyanin from wheat bran have been studied in various researches (Hu, et al., 2007; Liyana-Pathirana & Shahidi, 2005; J. Wang, et al., 2008) and it was found that 50-65% ethanol-water mixture was more effective. In one study, wheat bran (10%, 20% and 30% levels) and corn bran (10 and 20% levels) along with glucose oxidase and hexose oxidase were used to study its effect on rheological and bread making properties. Negative effects on dough and bread properties were observed in terms of Farinograph parameters. Wheat bran and corn bran that could be used at maximum level were found to be 20% and 10%, respectively (GÜL, et al., 2009). Different research done on treatment of wheat bran and their application in food products have been listed in table 5.

Table 5. Various research on wheat-bran treatment.

| Author name | Year | Research |
|---|---|---|
| Rasco, B.A., et al. | 1991 | Evaluation of enzyme and chemically treated wheat bran ingredients in yeast-raised breads |
| Wang, J., et al. | 2002 | Effect of the addition of different fibers on wheat dough performance and bread quality |
| Peressini, D. and Sensidoni, A. | 2009 | Effect of soluble dietary fiber addition on rheological and breadmaking properties of wheat doughs |
| GÜL, ÖZer, & DİZlek, 2009 | 2009 | Improvement of the wheat and corn bran bread quality by using glucose oxidase and hexose oxidase |
| Sahin, E. | 2011 | Utilization of wheat bran fiber in crackers |
| Favaro, L. et al. | 2012 | Processing wheat bran into ethanol using mild treatments and highly fermentative yeasts |
| Johansson, M. | 2012 | Dietary fiber composition and sensory analysis of heat treated wheat and rye bran |
| Nyombaire, G. | 2012 | Extrusion of wheat washed bran: Physicochemical and functional properties |
| Lehtinen, O.K | 2012 | Modifying wheat bran for food applications-Effect of wet milling and enzymatic treatment |
| Majzoobi, M. et al. | 2013 | Effect of different levels and particle sizes of wheat bran on the quality of flat bread |
| Reyes-Perez, F., et al. | 2013 | Estimated glycemic index and dietary fiber content of cookies elaborated with extruded wheat bran |
| Radenkovs, V. | 2014 | Application of enzymatic treatment to improve the concentration of bioactive compounds and antioxidant potential of wheat and rye bran |

Table 6. Polarity index of some solvents.

| Solvent | Polarity index |
|---|---|
| Water | 9.0 |
| 20% ethanol | 8.2 |
| 50% ethanol | 7.1 |
| 75% ethanol | 6.2 |
| 95% ethanol | 5.4 |
| Anhydrous ethanol | 5.2 |
| Methanol | 6.6 |
| Acetone | 5.4 |
| 75% Iso-propanol | 5.5 |
| 95% Iso-propanol | 4.5 |
| 100% Iso-propanol | 4.3 |

Source: Tang, et al. (2005).

## 2.7. Dough rheological properties

Most theories suggest that gluten is responsible for the rheological behavior of the dough. Well-developed dough can be elongated/stretched multiple times its original length provided that the elongation rate is not too high (Khan & Shewry, 2009). The hydration of flour leads to the formation of covalent and noncovalent interactions of wheat gluten proteins.

Various types of bread have been produced by the addition of bran but its effect on rheology have not been fully explored. Addition of wheat bran does not have good impact on dough rheology, texture and sensory attributes. Further, bread volume is also affected adversely. Though bran provides multiple health benefits, its use is not given much priority and they are usually used as fodder. Various efforts has been made to treat bran. Various studies have been done on enzymatic treatment of wheat bran to improve its quality (GÜL, et al., 2009; Laurikainen, et al., 1998; Lehtinen, 2012; Radenkovs, et al., 2014). In one study, wheat bran was ground to different particle size and their effect on rheological properties was studied (Decai Zhang & Moore, 1997). Similarly, research on changes in rheological and bread quality on addition of wheat bran can be found in various articles (Bonnand-Ducasse, et al., 2010; Gómez, et al., 2011; Gómez, et al., 2003; Özboy & Köksel, 1997).

Rheology is the study of deformation and flow of matter (Zaidel, et al., 2010). When a controlled and well-defined deformation or strain is applied to a material over a certain period of time and the derived force response or vice versa is measured, it gives an expression of material parameters like stiffness, hardness, viscosity and strength of the material. Thus rheology can either be used as a tool in process control and design or as a tool in the prediction and simulation of the materials to the flows and deformation conditions (Dobraszczyk & Morgenstern, 2003). The derivation of rheological properties of material depends upon the relationship between the stress on a material, the corresponding deformation or strain and time Strain is measured by deformation and stress can be compressive, tensile, or shear. Again all types of deformation divided into elastic deformation and flow. Elastic deformation can be recovered while incompletely recoverable deformations are linked with flow.

Rheological properties can be measured by carrying out many tests. For the cereal and baking industry, the most popular instruments are the Farinograph, mixograph, extensigraph, texturometer, TAXT2/Keiffer rig, etc. These tests can predict dough behavior in bakery industry at initial stage of the manufacturing process (Sanz Penella, et al., 2008). Most advanced rheological devices used to study the rheological behavior of dough during kneading and mixing are mixograph and the Farinograph (Hadnađev, et al., 2011).

2.7.1. Farinograph

The Farinograph was established in about 1930 and is used for measuring dough physical properties. It consists of a mixing bowl where two z-shaped blades rotating in opposite directions towards each other at varying speeds. Mixing bowls with 300 g, 50 g and 10 g capacity are available for use with the Farinograph. Water is added manually from a burette. The amount of water added depends upon the moisture content and water absorption of flour. After addition of water, the flour hydrates and dough forms followed by an increase in resistance on the mixing blades. The mixing curve rises to a maximum point and then slowly starts declining as the dough is weakened by over mixing and dough structure is ruptured. The maximum resistance is always centered on the 500 Brabender Unit (BU) line. The parameters obtained using a Brabender Farinograph are water absorption, dough development time or mixing time, mixing tolerance index and stability.

The amount of water added to enable the Farinograph curve to reach the 500 BU line is called water absorption and it is expressed as percentage of flour (14% moisture basis). Water absorption has gained the greatest practical value among the Farinograph parameter. Water absorption is important in evaluating the flour strength and the final product price calculations. Gluten and starch components mainly influence the Farinograph water absorption. High water absorption and low degree of softening indicates good quality flour, whereas a high water absorption with a high degree of softening indicates inferior quality flour. In general, water absorption values vary with the end product. For example its value for cookie and biscuit flour is about 50%, while its value is around 60% for bread flour.

Dough development time (DDT) which is often called mixing time or peak time is the time when the dough is optimally developed. It is the time between the origin of curve and when dough achieves the maximum consistency. Like water absorption, DDT also depends on the properties of the gluten quality, and starch granule. Furthermore, DDT increases with the increase in the proteolytic degradation of protein. It also increases with a decrease in the size of starch granule and the increase in the content of damaged starch due to the increase in specific surface area which absorbs water. The stability and the degree of softening are the gluten quality parameters which describe the viscoelastic properties of the formed gluten complex. The degree of softening is the distance between the center of the curve at the end of analysis time and the central line which passes through the maximum of the curve. In practice, higher stability and lower degree of softening indicate that dough will be more able to sustain long mechanical processing treatments. Increased degree of softening is particularly important indicator of proteolytic degradation of gluten.

Mixing tolerance index is measured in Brabender units. It is the difference between the top of the curve at which dough achieves the maximum consistency and the point on the curve after 5 minute. Dough stability represents the time during which the maximum dough consistency does not change or change very little. The quality number represents the area enclosed by line passing through the center of the Farinograph curve and the central line which passes through the maximum of the curve (500±10 BU).

Disulfide bonds form strong cross-linked within and between polypeptide chains resulting in the stabilization of hydrogen bonds and hydrophobic interactions. This bond is the main cause for the dough formation. Flour with high water absorption, longer mixing time and higher dough stability is considered to be of good quality. The Farinograph is also helpful in monitoring the effect of additives on dough quality. Thus it is an important technique to optimize flour processing in terms of standardization of variable flour quality produced from raw materials.

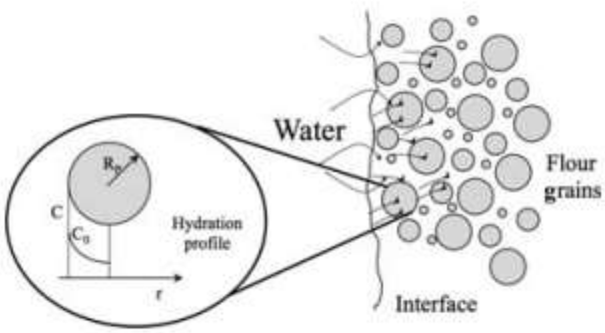


Figure 5. Flour hydration and water in grain diffusion.
Source: Migliori and Correra (2013).

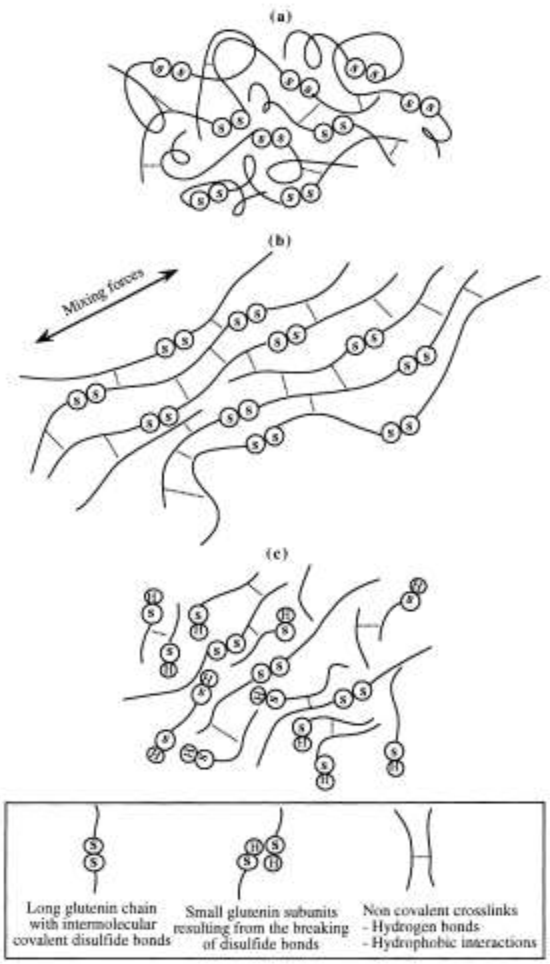


Figure 6. Molecular interpretation of the gluten development (a) beginning of the mixing, (b) optimum development, and (c) overmixing (Letang, et al., 1999).

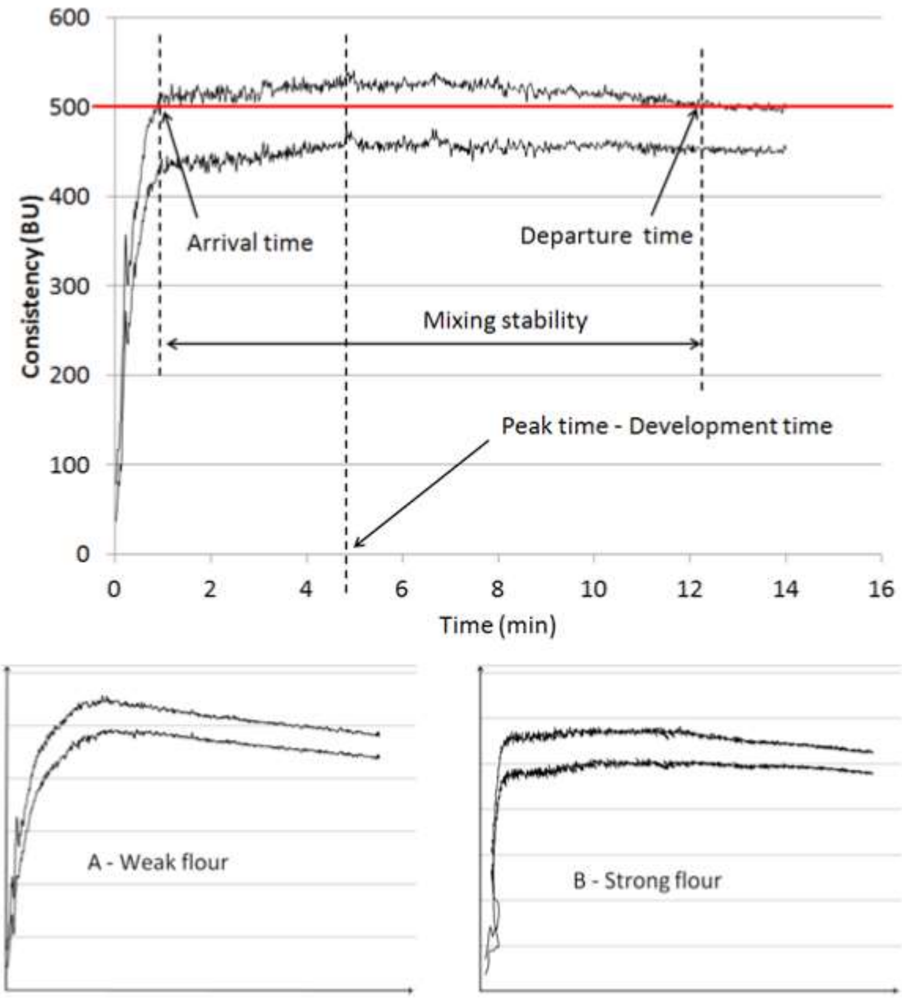


Figure 7. Typical Farinograph output.
Source: Krishnan and Darly-Kindelspire (2013).

2.7.2. Mixograph

The Mixograph is also one type of rheological device. This instrument also measures the resistance of dough during kneading, similar to a Farinograph. It was first developed in 1933. However, both processes differ in the kneading process and in the intensity of mechanical stress applied to the dough during analysis. It consists of a mixing bowl with four vertical pins revolving around three stationary pins in the bottom of the bowl. The Farinograph offers gentle kneading of dough by two z-shaped blades while the mixograph provides vigorous mixing (Mann, et al., 2008). The mixing process starts with hydration of the flour.

2.7.3. Texture analyzer

7.3.1. SMS/Kieffer dough and gluten extensibility rig

The Stable Microsystems (SMS) keiffer and dough dextensibilty rig, also called micro extensograph was developed by Keiffer (B Dunnewind, et al., 2003). This system works on the similar concept of extensograph. The only difference is that here, the sample is pulled upward (Zaidel, et al., 2010). The most important advantage of using Keiffer rig is that the sample used for the test can be as low as 0.4 g while for the extensograph about 150 grams of dough is required. Similarly, the Keiffer rig can be performed in different test speeds and the data can be directly stored into the computer (B. Dunnewind, et al., 2003).

7.3.2. One dimensional extensibility

The force used to rupture the dough is measured using Tortilla/Pastry Burst Rig. This technique has been used to measure the hardness/firmness of bread and the changes in their textural properties with time. This test was used to study the role of starch in flour tortilla staling using alpha-amylase where the deformation modulus, work, maximum force, and distance required to rupture the wheat tortilla were noted (Alviola & Waniska, 2008). Similarly, in another experimental approach, this test was used to study the rheology of wheat flour tortilla during storage. The parameters determined were the extensibility and the force used to rupture the tortilla (Bejosano, et al., 2005).

# CHAPTER 3. MATERIALS AND METHODOLOGY

## 3.1. Materials and methods

### 3.1.1. Selection and Collection of wheat

Five different varieties of HRSW were selected and collected from local breeders in SD. The five varieties were Advance, Brick, Select, Prevail, and Forefront.

### 3.1.2. Tempering of wheat

The moisture content of wheat kernel was determined using near-infrared (NIR) spectroscopy. Then the wheat was tempered to 15.5% moisture content by the addition of distilled water. For tempering water was added in stages; no more than 3 or 4 ml at a time. The formula used to calculate the amount of water added for tempering as per AACC method 26-95 was:

$$\text{ml}=\left[\left\{\frac{100\text{-}\%\text{moisture}}{100\text{-}15.5\%}\right\}-1\right]\times \text{grams of seed}$$

Wheat, after addition of water, was left for at least 24 hours at ambient conditions in a closed plastic jar to absorb the moisture for milling. The moisture content of the tempered wheat should not be greater than 17% or else it might clog the milling procedure.

### 3.1.3. Milling

The tempered wheat was milled through a Brabender Quadrumat Senior Mill. The output of the milling were bran and the white flour. Bran and flour from different wheat samples were stored in the freezer until use.

3.1.4. Size reduction of Bran

The bran size was reduced to fine particles by using the Restch mill using a 0.5 mm screen (Company: Restch GmbH & Co. Germany, Model: KG 5657HAAN1).

3.1.5. Extraction of wheat bran

The conventional extraction of wheat bran at room temperature was carried out using water and two portions of ethanol as solvent, 50% and 100%. Bran sample (200g) was extracted with 1000 ml of each of the solvent in a glass bowl for approximately one and half hours. After the extraction time, the extracts was filtered using 200 mesh and the solvent residue was washed using water. The treated and washed bran was then freeze dried. The dried bran was stored in an air tight bag in the freezer until use.

3.1.6. Minolta spectrophotometer

Color was measured with a Minolta spectrophotometer CM-580c (Konica Minolta Sensing America, Inc.) using the L-a-b opposable color scale.  The results of flour color measurement were reported in terms of a three dimensional color value based on the following rating scale:

L* value: Whiteness (100 for white and 0 for black)

a* value: Positive values for red color and negative values for blue color

b* value: Positive values for yellow color and negative values for blue color

L*a*b* reading of regular bran, solvent-treated bran (50% ethanol, 100% ethanol and water), whole wheat flour, and patent flour incorporated with treated bran was studied.

### 3.1.7. Wet gluten and gluten index

A Glutomatic system (Perten Instruments Huddinge, Sweden) was used to measure the wet gluten content (WG), dry gluten content (DG) and the gluten index (GI). The Glutomatic system included glutomatic, with kneader, attachment for washing chambers, tubing and submersible filter for solvent container, and electronics for 20-second dough mixing and 5-minute wash cycle for flour and 2-min wash, a stop, and then another 3 min for wheat meal. Sodium chloride solution (2%) was used as a wash solution. Standard washing chambers were provided with screen holders. Washing solvent was collected in a one liter plastic beaker. After the extraction, gluten was centrifuged at 6000±5 rpm. The wet gluten was dried to obtain the dry gluten content. The total dry gluten content was expressed as percent of sample, and the gluten index (GI) was expressed as percentage of wet gluten remaining on the sieve after centrifuging.

### 3.1.8. Proximate analysis

The five varieties of wheat flour and flour incorporated with treated bran was analyzed for fat, protein, moisture, carbohydrate and ash by standard methods.

#### 3.1.8.1. Moisture

Moisture content was measured using the hot air oven (Model Labline, Inc Chicago, IL, U.S.A) by AACC (1999) method. The temperature used was $135^0$C. The moisture content of the sample was measured by the formula:

$$\%\text{Moisture} = \left(\frac{M_w}{M_{\text{sample}}}\right) \times 100$$

where, $M_w$ is the mass of water and $M_{sample}$ is the initial weight of the sample

3.1.8.2. Fat

Continuous soxhlet extraction was used for fat extraction. An automated Soxhlet extraction method using petroleum ether by AACC (1999) method was used. At the end of the extraction process, the solvent was evaporated by placing the receiving flask overnight at room temperature and the weight of lipid in the flask ($M_{lipid}$) was measured. The percentage of lipid present in the initial sample ($M_{sample}$) was then calculated using the following formula:

$$\%\text{Lipid} = \frac{M_{lipid}}{M_{sample}} \times 100$$

3.1.8.3. Protein

Protein analysis was done by using the combustion method 46-30 (AACC, 1999) with CE Elantech Flash EA 1112 (ThermoFinnigan Italia S.p.A., Rodano (MI) Italy). Percent nitrogen was converted into % protein by using a conversion factor of 5.7. Protein content of flour incorporated with treated bran was studied.

3.1.8.4. Ash

Ash content was determined by the AACC (1999) method using a muffle furnace (Lindberg/Blue 1100°C Box furnace BF 51800 series Ashville, NC) at 525 °C. The sample was left at $525^{0}$C continuously for 5 hours. The sample was weighed before and after ashing to calculate the ash content in the sample.

$$\%\text{Ash(dry basis)} = \frac{\text{(Weight after ashing - weight of empty crucible)}}{\text{Original sample weight*dry matter coefficient}} \times 100$$

3.1.8.5. Total carbohydrate

Total carbohydrate was calculated by difference by AOAC (1990) method (Helrich, 1990). Percentage of protein, fat, ash, and moisture content of the sample were determined individually, summed and subtracted from 100.

Total carbohydrate = $100 - (\text{Protein\%} + \text{Fat\%} + \text{Ash\%} + \text{Moisture\%})$

3.1.9. Total dietary fiber (TDF)

TDF analysis of whole wheat flour was done using a gravimetric technique. The method used was a simplified modification of AACC total dietary fiber method, 32-05.01. Here, whole wheat flour (1 g) was subjected to sequential enzymatic digestion by three different enzymes, heat-stable alpha-amylase, protease and amyloglucosidase. The entire procedure is presented in figure 8.

Total dietary fiber was determined using the formula:

Dietary fiber (%) = $\left(\frac{\frac{R1+R2}{2}-p-A-B}{\frac{m1+m2}{2}}\right) * 100$

where, R1=Residue weight 1 from m1; R2=Residue weight 2 from m2; m1=sample weight 1; m2=sample weight 2; A=ash weight from R1; p=protein weight from R2; and

B=Blank=$\left(\frac{BR1+BR2}{2}\right) - Bp - BA$

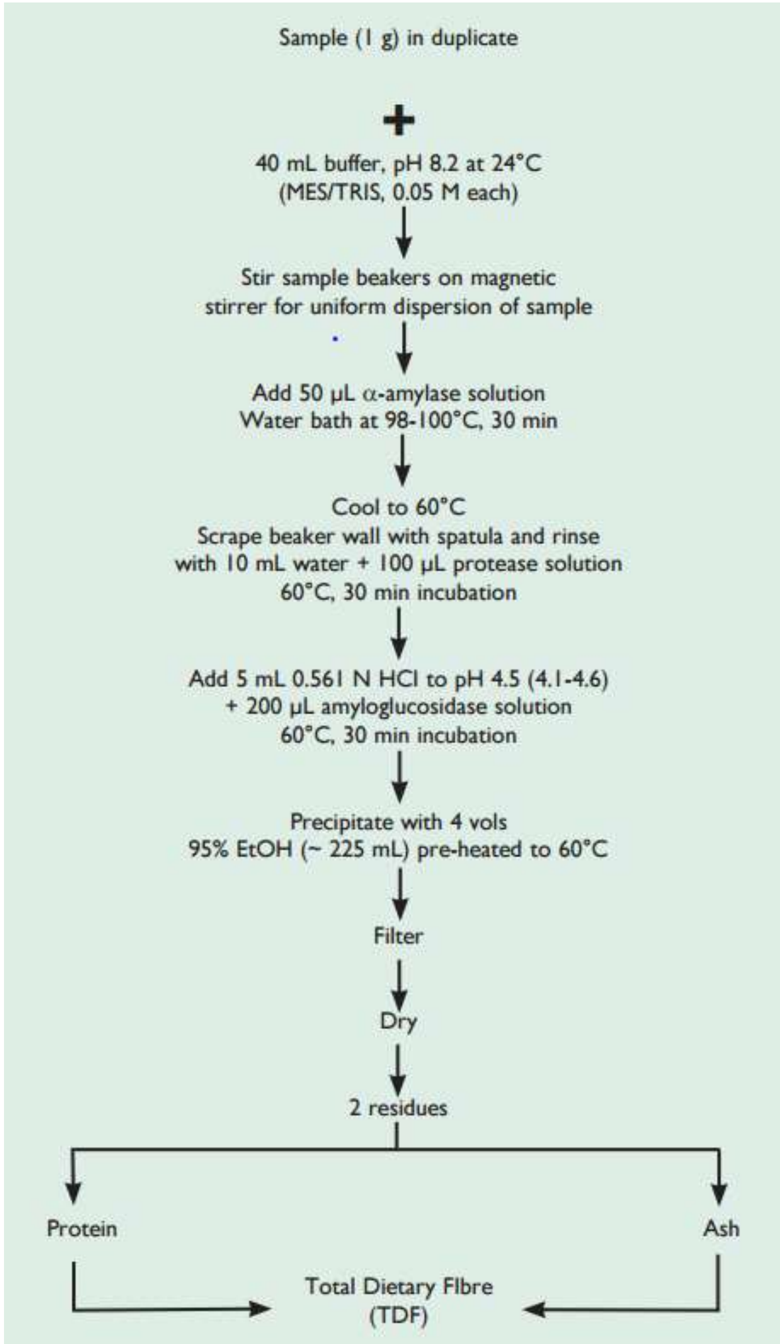


Figure 8. Analytical scheme of total dietary fiber determination procedure.

3.1.10. Development of whole wheat flour

Treated bran was mixed with the patent flour using a twin-shelled dry blender (Peterson Kelly Co. Inc. Stroudsburg, PA) to obtain the whole wheat flour. Bran incorporation was not less than 15%.

3.1.11. Particle size measurement of whole wheat flour

The particle size measurement of all the samples was done using a Ro-Tap Test Sieve Shaker (W.S. Tyler, Model: RX-29, Serial: 12266). The particle size was analyzed using USA standard testing sieve with mesh size 60 (250 µm), 80 (180 µm), 100 (150 µm), 200 (75 µm) and below 200 (>75 µm). The particle size was expressed as the percentage of particles retained on each sieve. All measurements were done in duplicate.

3.1.12. Dough rheological properties

The Farinograph E (CW Brabender Instruments, Inc., South Hackensack, NJ) was used to study the rheological properties of dough developed from patent flour incorporated with treated bran. The parameters determined by Brabender Farinograph were water absorption (WA), dough development time (DDT), dough stability (DS), mixing tolerance index (MTI), and the time to breakdown (BT). The analysis was carried out for whole wheat flour and the blend of patent flour with treated bran. The instrument was turned on and the amount of weight displayed on the computer screen was placed in the Farinograph mixing bowl and water was added from a burette. When water was added, flour was hydrated. As the mixing process proceeded, gluten, which is the main protein present in wheat flour, started to form a network by the formation of disulfide bonds. The consistency of dough increased with the degree of dough development until it reached a maximum point which was called the peak value or the dough development time. As the mixing time increased, the dough weakened because of the breakage of disulfide bonds and the formation of shorter protein fibrils. This made the dough more sticky. The curve was centered on the 500-Barbender Unit (BU) line ±20 BU by adding the appropriate amount of water until the curve left the 500-BU line.

#### 3.1.13. Dough Extensibility

Dough extensibility properties were studied using the SMS/Kieffer Dough Extensibility Rig fitted onto a TA.XT.*plus* texture Analyzer (Texture Technologies Corp., Scarsdale, NY). Ten grams of the flour was taken and the dough was mixed to optimum consistency. Then the dough was placed in a Teflon mold which shaped the dough into strips of the same dimension. The dough and the Teflon mold were coated with mineral oil to prevent stickiness and drying of the dough. The dough strips were allowed to rest for 40 minutes. Dough strips were then clamped into the Kieffer rig platform. The hook moved upward stretching the dough until it was broken. The resistance of a dough to stretching was measured and recorded. The parameters determined were dough maximum resistance ($R_{max}$), dough extensibility ($E_{max}$), area under the curve, initial slope of the curve ($E_i$) and extensibility at maximum resistance ($E_{Rmax}$).

#### 3.1.14. Product development

Among the five different varieties of wheat samples only one variety (Prevail) was processed into a finished product. Tortilla was made from the whole wheat flour and the flour fortified with 15% of treated bran. The treated bran samples were milled to small particle size before adding them to the patent flour. Bran size was reduced using a

Restch Mill (Restch GmbH & Co.KG, Germany) operated at 20,000 rpm using a 0.5 mm sieve. Bran was mixed with the patent flour using a blender (Blend Master, Patterson-Kelly, Harsco, East Stroudsburg, PA, USA) for 45 minutes.

A Hot-press tortilla maker was used to make tortillas using a standard formula given in the table 7. At first, the dry materials were mixed in a mixer at low speed for about 2 minutes. Shortening was then added and mixed for 6 minutes. Finally, water was added and mixed further for 4 minutes at medium speed. The dough was allowed to rest for 10 minutes in a covered container. The dough was divided manually into 20 g dough balls and allowed to rest for 10 minutes. After resting, a tortilla hot press (Dual Heat Doughpro Tortilla Press, Propress Corp., Paramount, CA, USA) was used to press the dough into a specific thickness. The temperature of both the upper and lower plate was set at $100^0$C and the press time was set for 15 seconds at the thin setting. The tortilla was pressed twice for 15 seconds. Baking the tortilla was then done on the heated metal plate surface of an oven at $150^0$C for 35 seconds on each side. This was followed by cooling of tortillas in a metal tray for five minutes.

3.1.15. Evaluation of tortilla properties

Two tortillas from each sample were selected and measured for color. Color was measured with a Minolta spectrophotometer CM-580c (Konica Minolta Sensing America, Inc) using L-a-b opposable color. Here L* is the measure of lightness, a* is the measure of greenness to redness, and b* is the measure of blueness to yellowness.

Table 7. Hot-press whole wheat tortilla formulation

| Ingredient | Amount (g) | % Flour Basis |
|---|---|---|
| Flour (14% moisture basis) | 100 | 100 |
| Salt | 1.4 | 1.4 |
| Baking powder | 0.8 | 0.8 |
| Vegetable oil | 8 | 8 |
| Water | Adjusted | - |

Source: Guo, et al. (2003);Modified

3.1.16. One dimensional extensibility using Tortilla/Pastry burst rig

An acrylic probe with 7/16 inch diameter was attached to the analyzer arm. A tortilla was fixed on the analyzer platform. Uniaxial extensibility test was performed with "return to speed" option with compression force and trigger force of 5 g. Pretest, test and post-test speeds were maintained to 1mm/s, 1 mm/s and 10 mm/s, respectively.

3.1.17. Sensory analysis

Sensory analysis of tortilla with treated wheat bran was done using untrained panelists. 12 panelists were asked to evaluate the product in terms of appearance, aroma, texture, taste, and overall acceptability. The panel members included faculty and graduate students in a Food Processing class. A five-point hedonic rating scale (1-dislike extremely to 5-like extremely) was used. Only one variety of wheat was used for the sensory evaluation at a given session. Samples were randomly coded and served individually along with distilled water.

3.1.18. Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was done on all data by performing SPSS software using Type I error where $\alpha$ level was 0.05, by analysis of variance (ANOVA) to find if there was significant differences among the treatments. Post-hoc Duncan test was done to determine where the differences occurred. Pearson's correlation coefficients (r) were also computed to understand the relationships between different variables.

3.1.19. Experimental design

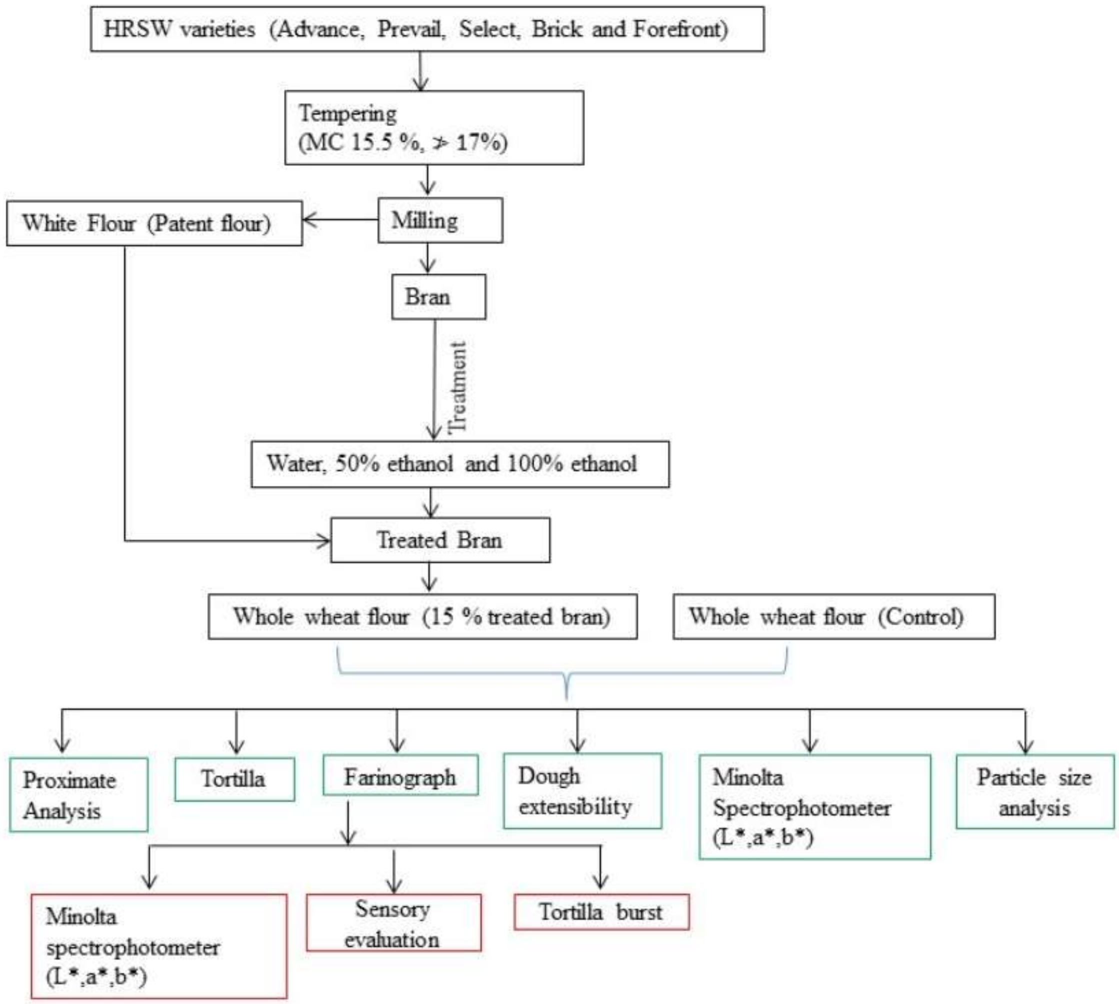


Figure 9. Experimental design employed in the processing and analysis of wheat samples.

# CHAPTER 4. RESULTS AND DISCUSSION

## 4.1. Tempering and milling

The purpose of tempering was to toughen the bran so that it could be separated easily from the endosperm in the milling and sifting operation and to secure the correct percentage of moisture in the finished product. The moisture content of the wheat in our study ranged from 12.0-13.4% and the moisture content of the tempered wheat ranged from 15.1-15.9%. After milling, the moisture content of white flour ranged from 11.11-11.56%. Usually, the moisture content of white flour is kept below 14% because at 14% moisture, fungal growth may take place and the flour will deteriorate early (Butt, et al., 2004). Yield of white flour ranged from 58.6% to 64.5%, and for the bran fraction, it was 9.45% to14.45%.

Table 8. Moisture content and yield % of five HRSW cultivars

| Wheat cultivars | M.C of wheat (%) | M.C of tempered wheat (%) | M.C of flour (%) | White flour yield (%) | Bran yield (%) |
|---|---|---|---|---|---|
| Advance | 12.57 | 15.88 | 11.48 | 59.87 | 13.20 |
| Prevail | 12.92 | 15.75 | 11.31 | 63.61 | 14.45 |
| Select | 12.00 | 15.12 | 11.11 | 59.45 | 9.45 |
| Brick | 12.46 | 15.43 | 11.21 | 64.5 | 12.13 |
| Forefront | 13.39 | 15.44 | 11.56 | 58.6 | 11.75 |

M.C. = Moisture content


Figure 10. (a) Tempering of whole wheat (b) wheat milling (c) white flour (left) and wheat bran (right) obtained after milling.

4.2. Particle size

All of the flour samples were subjected to particle-size measurement using a Tyler Ro-Tap Sieve Shaker (W.S. Tyler Co., Mentor, Ohio). Wheat flour particle size was studied using six sifter sizes: 40, 60, 80, 100, 200 and >200 mesh. Particle-size measurement on two commercially available whole wheat flours was also done.

Table 9-11 provide the particle distribution of flour. As shown in table 9, more flour was retained on the 100 mesh sieve for commercial whole wheat flour, while for all other flour samples, flours retained on 200 and >200 mesh was the highest. Commercial King Arthur whole wheat flour had significantly greater ($p<0.05$) retention on 60 and 100 mesh sieve. Schmiele, et al. (2012) also found larger particles for whole wheat flour and showed that approximately 87% passed through the 32 mesh sifter. In our study, approximately 90% passed through the 40 mesh sifter. Schmiele, et al. (2012) also explained that 95% of the wheat flour should pass through a sieve with size 60 mesh. However, because of presence of bran fiber, which show greater resistance to grinding, whole grain wheat flour had larger particle size (Schmiele, et al., 2012). Among the five cultivars, Prevail had more coarse bran particle size than other cultivars, since significantly higher flour retention were obtained in 40, 60 and 80 mesh sieve as shown in the table 9. Similarly, in the case of treatments used, flour incorporated with water washed bran had higher retention on 40 mesh sieve and the value was significantly different than others. Water washed bran swelled and might have been larger compared to bran treated with ethanol.

Wheat bran is hygroscopic in nature. Various studies suggest that wheat bran hydration properties are dependent on its particle size. The water-binding capacity of wheat bran decreased with decreasing particle size (Auffret, et al., 1994; Jacobs, et al., 2015; D Zhang & Moore, 1999). In some studies, Farinograph water absorption was found to be unaffected by bran particle size (Auffret, et al., 1994; Jacobs, et al., 2015; D Zhang & Moore, 1999). However, Albers, et al. (2009) reported decreased Farinograph water absorption with decreasing particle size, and in contrast to this, the study by Sanz Penella, et al. (2008) suggested increased Farinograph water absorption with higher fine bran addition and decreased water absorption with the addition of coarse bran. Finer particle size results in increased surface area of the bran and this might have been responsible for the increased water absorption.

Wheat bran particle size has been found to influence sensory attributes of bread. D Zhang and Moore (1999) study showed significant effect of wheat bran particle size on specific volumes and sensory quality of bread. They reported higher loaf volume for bread which contained wheat bran with 415 µm (~ 40 mesh sieve) particle size.

Table 9. Particle size comparison among the whole wheat flours from the five HRSW cultivars and commercial whole wheat flours.

| Whole wheat flours | 40 mesh | 60 mesh | 80 mesh | 100 mesh | 200 mesh | >200 mesh |
|---|---|---|---|---|---|---|
| Commercial | | | | | | |
| Dakota | 4.30d | 7.60b | 19.10a | 20.70b | 24.0c | 22.80c |
| King Arthur | 8.30c | 13.70a | 15.00b | 33.50a | 20.90d | 7.70d |
| Cultivars | | | | | | |
| Advance | 9.20b | 5.030e | 5.25f | 15.77cd | 33.5b | 26.93bc |
| Prevail | 10.57a | 5.95c | 8.23c | 14.07de | 32.55b | 26.25bc |
| Select | 7.99c | 5.69d | 6.28d | 17.33c | 19.87d | 42.00.a |
| Brick | 9.47b | 6.03c | 6.15d | 12.73e | 36.87a | 26.7bc |
| Forefront | 8.02c | 5.59d | 5.71e | 13.63e | 34.93ab | 31.55b |

Values followed by the same letter in the same row are not significantly different ($p<0.05$).

Table 10. Comparison of particle size among the commercial wheat flour and the wheat flour incorporated with the treated bran.

| | 40 mesh | 60 mesh | 80 mesh | 100 mesh | 200 mesh | >200 mesh |
|---|---|---|---|---|---|---|
| Commercials | | | | | | |
| Dakota | 4.30e | 7.60c | 19.10a | 20.70b | 24.00c | 22.80b |
| King Arthur | 8.30c | 13.70a | 15.00b | 33.50a | 20.90d | 7.70c |
| Treatments | | | | | | |
| Control (WWF) | 7.27d | 10.74b | 14.72b | 20.62b | 24.28c | 18.63b |
| Water | 10.38a | 4.20d | 4.31c | 12.51c | 32.06b | 35.19a |
| Ethanol 50% | 9.61b | 3.77e | 2.91e | 13.09c | 34.24ab | 34.87a |
| Ethanol 100% | 8.95c | 3.94e | 3.35d | 12.60c | 35.59a | 34.05a |

Values followed by the same letter in the same column are not significantly different ($p<0.05$).
Control (WWF) = Whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, and ethanol 100% = flour incorporated with bran treated with 100% ethanol.

Table 11. Particle size of the wheat flours incorporated with the treated bran and whole wheat flours of five HRSW.

| Cultivars | Treatments | 40 mesh | S.D. | 60 mesh | S.D. | 80 mesh | S.D. | 100 mesh | S.D. | 200 mesh | S.D. | >200 mesh | S.D. | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Advance | Ethanol 100% | 10.90 | 0.14 | 3.60 | 0.00 | 2.00 | 0.00 | 9.90 | 0.14 | 38.50 | 0.14 | 32.90 | 0.71 | 2 |
| | Ethanol 50% | 9.70 | 0.14 | 3.80 | 0.00 | 2.30 | 0.14 | 12.70 | 0.42 | 39.50 | 0.14 | 30.40 | 0.57 | 2 |
| | Water | 11.60 | 0.00 | 3.80 | 0.00 | 2.40 | 0.28 | 9.50 | 0.42 | 37.50 | 0.14 | 33.90 | 0.42 | 2 |
| | Control (WWF) | 4.60 | 1.98 | 8.90 | 0.14 | 14.30 | 0.42 | 31.00 | 1.41 | 18.50 | 2.12 | 10.50 | 14.85 | 2 |
| Previal | Ethanol 100% | 8.90 | 0.99 | 3.90 | 0.14 | 4.50 | 0.14 | 11.20 | 0.28 | 39.40 | 1.13 | 30.50 | 1.27 | 2 |
| | Ethanol 50% | 9.10 | 0.42 | 4.30 | 0.14 | 5.00 | 0.28 | 13.60 | 0.28 | 36.70 | 0.42 | 28.30 | 2.12 | 2 |
| | Water | 11.30 | 0.14 | 4.10 | 0.14 | 9.70 | 0.42 | 19.50 | 2.12 | 29.30 | 0.71 | 23.60 | 1.98 | 2 |
| | Control (WWF) | 13.00 | 0.00 | 11.50 | 0.14 | 13.70 | 0.42 | 12.00 | 1.41 | 24.80 | 1.41 | 22.60 | 3.68 | 2 |
| Select | Ethanol 100% | 7.43 | 0.10 | 4.30 | 0.14 | 4.03 | 0.01 | 17.42 | 0.88 | 20.77 | 4.57 | 45.13 | 5.56 | 2 |
| | Ethanol 50% | 8.59 | 0.13 | 3.96 | 0.06 | 2.82 | 0.03 | 14.10 | 0.59 | 16.10 | 4.07 | 53.75 | 3.41 | 2 |
| | Water | 11.39 | 0.16 | 3.93 | 0.01 | 2.88 | 0.00 | 14.08 | 0.48 | 22.06 | 4.04 | 45.16 | 3.71 | 2 |
| | Control (WWF) | 4.58 | 0.14 | 10.57 | 0.10 | 15.39 | 0.13 | 23.70 | 4.78 | 20.54 | 1.19 | 23.96 | 5.77 | 2 |
| Brick | Ethanol 100% | 9.50 | 0.14 | 4.00 | 0.00 | 3.00 | 0.00 | 11.90 | 0.14 | 40.60 | 0.85 | 28.80 | 0.85 | 2 |
| | Ethanol 50% | 10.30 | 0.14 | 3.60 | 0.00 | 2.20 | 0.00 | 10.40 | 0.57 | 41.60 | 0.57 | 30.00 | 1.41 | 2 |
| | Water | 10.20 | 0.00 | 4.10 | 0.14 | 2.80 | 0.00 | 9.10 | 0.42 | 38.60 | 0.57 | 33.30 | 0.99 | 2 |
| | Control (WWF) | 7.90 | 0.14 | 12.40 | 0.28 | 16.60 | 0.57 | 19.50 | 4.10 | 26.70 | 0.14 | 14.70 | 4.38 | 2 |
| Forefront | Ethanol 100% | 8.03 | 0.07 | 3.91 | 0.01 | 3.22 | 0.03 | 12.60 | 0.28 | 38.70 | 0.51 | 32.92 | 0.31 | 2 |
| | Ethanol 50% | 10.36 | 0.08 | 3.20 | 0.00 | 2.22 | 0.03 | 14.67 | 0.58 | 37.29 | 0.35 | 31.88 | 0.62 | 2 |
| | Water | 7.40 | 0.06 | 4.98 | 0.03 | 3.76 | 0.00 | 10.35 | 1.15 | 32.86 | 1.10 | 40.00 | 0.48 | 2 |
| | Control (WWF) | 6.27 | 0.07 | 10.27 | 0.01 | 13.62 | 0.08 | 16.88 | 2.09 | 30.86 | 4.16 | 21.38 | 6.34 | 2 |

Control (WWF) = Whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, and ethanol 100% = flour incorporated with bran treated with 100% ethanol, and S.D. = standard deviation.

Figure 11. Ro-Tab sieve shaker for particle analysis.

4.3. Proximate analysis of whole wheat flour

Table 12-13 provide proximate composition of the five HRSW varieties and the wheat flours incorporated with the treated brans. Here, the whole wheat flours obtained from direct milling of whole wheat were used as the control for comparison. The hot air oven method was used to measure the moisture content. A muffle furnace was used for the ash content determination. An ether extraction method was employed for fat analysis. Dumus combustion method was used for protein analysis. Carbohydrate content was determined by difference.

The moisture content of all flours ranged from 9.9% to 11.5%. The lowest moisture content was observed for the control (WWF). The standard deviation for all samples were below 1 which means deviation from the mean was minimal. The moisture content of Prevail cultivar was the highest (10.8%) followed by Brick cultivar (8.4%), and those values were significantly different from all other cultivars. Similarly, the moisture content of Advance, Select and Forefront flour were the lowest with no significant differences. Among the four treatments, control (WWF) had the lowest moisture content (9.8%) while the flour incorporated with 100% ethanol treated bran had the highest (10.6%) and the values were significantly different from the other samples at $p<0.05$. Moisture content of the flour mixed with water washed bran and flour mixed with ethanol 50% treated bran were not significantly different. Determination of the moisture content of flour is an important parameter in analyzing the flour quality since this data is used for other tests. Lower the moisture content of the flour was conducive to higher the storage stability.

The ash content ranged from 1.3% to 1.7%. Ash content as a dry basis for Brick, Forefront and Advance cultivars were found to be higher compared to other cultivars with no significant differences, while Prevail and Select cultivar had comparatively lower ash content. There was no significant difference in ash content among control (WWF) and the flour incorporated with bran treated with ethanol 50%. There was also no variation in ash content among the flour incorporated with water washed and 100% ethanol washed bran. Ash content of 98 HRSW were determined by E. B. Maghirang, et al. (2006) and the average value obtained was 1.67% with the minimum value of 1.27% and the maximum of 1.93% (E. Maghirang, et al., 2006). Seyer and Gélinas (2009) also studied the ash content on four different types of HRSW cultivar and the value was found to be 1.81%-1.93%. These values were higher than our results. This may be because minerals content vary according to wheat cultivar and the environmental conditions in which they are grown (Oury, et al., 2006; Zhao, et al., 2009).

Crude fat percentage value for all the flour samples ranged between 1.0-2.1%. The lowest fat content was obtained for flours incorporated with 50% ethanol treated bran. Higher fat content was obtained for the control (1.74%). There was significant difference among the fat content of five cultivars and treatments. Fat content of Forefront cultivar was found to be the lowest while Advance had the highest fat content. Flour incorporated with 50% ethanol treated bran was found to have significantly less fat content (1.23%) followed by flour incorporated with 100% ethanol treated bran (1.51%). This means ethanol 50% was more effective to remove fat from wheat bran than ethanol 100% and water. A 50% ethanol solution is the mixture of both polar and nonpolar solvents in equal proportion and it has been suggested that more polar solvents are required to dissociate lipids from protein components (Hlynka, 1964). Tsen, et al. (1962) used a mixture of ethyl alcohol, chloroform and water in the ratio of 2:1:0.8 for the effective extraction of lipids from ground wheat samples and the requirement of water for removal of additional lipids was proved. This supported the finding that 50% ethanol was more effective in removing fat than 100% ethanol.

The protein content for all the flour samples ranged between 12.22-16.47%. The control (WWF) had the highest protein content, ranging from 13.79-16.47%. Advance cultivar had the lowest protein content of 13.79% and Select cultivar had the highest protein content of 16.47%. White flour protein content ranged from 12.2-14.5%. Protein content of wheat varieties differs with location and environmental condition. In the 2012 Research Report by South Minnesota Wheat Research, the protein content of Advance, Brick, Forefront and Select cultivars at two southern MN Sites were found to be 14.5%, 15.4%, 14.9%,and 15.4% and 14.2%, 15.4%, 15.4%, and 15%, respectively (Rot & Rot). Similarly, in the study by Seyer and Gélinas (2009), protein content of four different hard red spring cultivar were found to be 12.2%-13.5%. The protein value from our study was also found to be in the similar range. In the case of treatments used, protein content of flour incorporated with water treated bran was higher than the flour incorporated with ethanol treated bran. The least protein content was found for flour incorporated with 100% ethanol treated bran. Wheat protein content is an important factor in baking and in the production of other wheat based products like pasta and noodles. The protein content varies widely depending on the type of wheat, fertilizers used and growing conditions.

Total carbohydrate was determined by difference by the AOAC (1990) method. Here, the sum of moisture%, fat%, protein%, and ash% was subtracted from 100% to get the total carbohydrate content. Application of the same method to determine carbohydrate can be found in various literature (Garg & Dahiya, 2003; Schmiele, et al., 2012). Carbohydrate content of all five cultivars were significantly different to each other. Forefront had significantly highest carbohydrate content, followed by Advance cultivar, while Prevail had significantly lower carbohydrate content. In the case of treatments used, there was no significant difference between the carbohydrate content of the flour incorporated with bran treated with 50% and 100% ethanol. Control (WWF) had significantly lower carbohydrate content. Forefront had significantly lower moisture and protein content which had resulted in significantly higher carbohydrate content of Forefront. In the same way, since Prevail had highest moisture and protein content, its carbohydrate content was lower.

Table 12. Comparison of proximate composition of whole wheat flours and wheat flours made from the incorporation of treated bran.

| Whole wheat flours | Moisture (%) | Ash (%) | Fat (%) | Protein (%) | Carbohydrate (%) |
|---|---|---|---|---|---|
| Cultivars | | | | | |
| Advance | 10.25c | 1.56a | 1.76a | 14.03b | 72.4b |
| Prevail | 10.81a | 1.46b | 1.43d | 14.8a | 71.5e |
| Select | 10.17c | 1.46b | 1.64b | 14.65a | 72.07c |
| Brick | 10.41b | 1.55a | 1.58c | 14.64a | 71.8d |
| Forefront | 10.27c | 1.55a | 1.31e | 14.16b | 72.7a |
| Treatments | | | | | |
| Control (WWF) | 9.98c | 1.63a | 1.74a | 15.24a | 71.42c |
| Water | 10.49b | 1.32c | 1.68b | 14.48b | 72.03b |
| Ethanol 50% | 10.43b | 1.62a | 1.23d | 14.32c | 72.4a |
| Ethanol 100% | 10.62a | 1.49b | 1.51c | 13.79d | 72.6a |

Values followed by the same letter in the same column are not significantly different ($p<0.05$).
Control (WWF) = whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, and ethanol 100% = flour incorporated with bran treated with 100% ethanol.

Table 13. Proximate composition of ground whole wheat flour and flour made from the incorporation of treated brans.

| | | Moisture % | | Ash % | | Fat % | | Protein % | | Carbohydrate% | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivars | Treatments | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Advance | Ethanol 100% | 10.97 | 0.05 | 1.53 | 0.01 | 1.67 | 0.04 | 13.55 | 0.03 | 72.27 | 0.03 |
| | Ethanol 50% | 10.18 | 0.03 | 1.70 | 0.08 | 1.14 | 0.03 | 13.88 | 0.12 | 73.11 | 0.14 |
| | Water | 10.74 | 0.04 | 1.48 | 0.03 | 2.03 | 0.06 | 14.89 | 0.18 | 70.86 | 0.22 |
| | Control (WWF) | 9.10 | 0.08 | 1.53 | 0.01 | 2.21 | 0.13 | 13.79 | 0.27 | 73.38 | 0.21 |
| Brick | Ethanol 100% | 10.22 | 0.01 | 1.48 | 0.09 | 1.73 | 0.01 | 13.51 | 0.02 | 73.07 | 0.06 |
| | Ethanol 50% | 11.00 | 0.06 | 1.69 | 0.04 | 1.25 | 0.02 | 14.57 | 0.15 | 71.50 | 0.07 |
| | Water | 10.32 | 0.12 | 1.38 | 0.02 | 1.61 | 0.04 | 14.86 | 0.06 | 71.83 | 0.13 |
| | Control (WWF) | 10.12 | 0.04 | 1.66 | 0.01 | 1.72 | 0.06 | 15.61 | 0.18 | 70.89 | 0.08 |
| Forefront | Ethanol 100% | 10.50 | 0.00 | 1.55 | 0.00 | 1.31 | 0.03 | 12.91 | 0.07 | 73.73 | 0.04 |
| | Ethanol 50% | 10.11 | 0.04 | 1.64 | 0.02 | 1.17 | 0.04 | 14.06 | 0.25 | 73.02 | 0.36 |
| | Water | 10.28 | 0.19 | 1.31 | 0.01 | 1.42 | 0.04 | 14.38 | 0.02 | 72.61 | 0.20 |
| | Control (WWF) | 10.17 | 0.01 | 1.69 | 0.10 | 1.32 | 0.01 | 15.32 | 0.32 | 71.50 | 0.42 |
| Prevail | Ethanol 100% | 10.71 | 0.25 | 1.54 | 0.02 | 1.34 | 0.07 | 14.77 | 0.11 | 71.65 | 0.06 |
| | Ethanol 50% | 10.70 | 0.06 | 1.54 | 0.10 | 1.12 | 0.04 | 15.01 | 0.04 | 71.63 | 0.04 |
| | Water | 11.29 | 0.16 | 1.18 | 0.05 | 1.73 | 0.03 | 14.43 | 0.18 | 71.37 | 0.31 |
| | Control (WWF) | 10.54 | 0.12 | 1.53 | 0.18 | 1.52 | 0.04 | 15.02 | 0.04 | 71.39 | 0.06 |
| Select | Ethanol 100% | 10.71 | 0.00 | 1.39 | 0.10 | 1.51 | 0.04 | 14.25 | 0.20 | 72.14 | 0.34 |
| | Ethanol 50% | 10.16 | 0.09 | 1.51 | 0.02 | 1.48 | 0.03 | 14.09 | 0.28 | 72.77 | 0.19 |
| | Water | 9.86 | 0.10 | 1.25 | 0.11 | 1.62 | 0.01 | 13.82 | 0.05 | 73.45 | 0.05 |
| | Control (WWF) | 9.96 | 0.06 | 1.71 | 0.01 | 1.93 | 0.08 | 16.47 | 0.13 | 69.93 | 0.15 |

Control (WWF) = whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, and ethanol 100% = flour incorporated with bran treated with 100% ethanol, and S.D. = standard deviation.

4.4. Total Dietary fiber (TDF)

TDF values for the five HRSW are provided in table 14. The TDF values ranged between 7.56-9.06%. In the study by Andersson, et al. (2013), 129 winter wheat samples were analyzed for TDF content and the TDF value ranged from 11.5-15.5%. TDF content of WWF obtained from our study were found to be lower in value.

CODEX defines dietary fiber as carbohydrate polymers with ten or more monomeric units, which cannot be hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories:

- Edible carbohydrate polymers naturally occurring in the food as consumed,
- Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities and synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities (Pomeranz, 1988a).

Most of the research reports increased water absorption with increase in fiber (Sanz Penella, et al., 2008; Seyer & Gélinas, 2009; Van Hung, et al., 2007; D Zhang & Moore, 1999). Though Select cultivars had the highest TDF (9.06%), Farinograph water absorption for Select flour was not the highest. In fact, Brick cultivar was found to have highest water absorption but the TDF of Brick cultivar was found to be 8.05%. This may be because TDF is not only the parameter affecting water absorption. Water absorption is affected by various other parameters such as gluten quality, protein and the amount of starch present.

Table 14. Total Dietary Fiber (TDF) of five HRSW cultivars.

| Cultivars | TDF % (db) |
|---|---|
| Select | 9.06a |
| Advance | 8.63a |
| Prevail | 8.20a |
| Forefront | 7.56a |
| Brick | 8.05a |

TDF=Total dietary fiber

### 4.5. Color measurement using Minolta Spectrophotometer

#### 4.5.1. Bran color

Tables 15-16 provide the overall color of the wheat bran after water and ethanol treatment. The color parameters were expressed in terms of L* (lightness), a* (redness) and b* (yellowness). L* value ranged from 61.22 to 65.84. The highest L* (64.57) was obtained for Forefront bran while the lowest L * (61.35) value was obtained for the Advance bran. Similarly, Forefront bran had the lowest a* and the highest b*. The a* for the Forefront bran was significantly different to other values. This means Forefront bran had the highest brightness, lower redness and higher yellowness. Advance and Brick bran had lower brightness with L* value of 61.35 and 61.91 respectively. a* for Advance and Brick bran were higher with the value of 6.22 and 6.1, respectively and b* value were lower with 16.54 and 16.72 values. This implies that Advance and Brick bran had lower brightness, higher redness and lower yellowness. The b* value was highest for Prevail bran which shows Prevail bran was comparatively more yellow in color.

The bran samples from five different wheat varieties were washed with 50% ethanol, 100% ethanol and water. The average L* value for water treated, 50% ethanol treated and 100% ethanol treated bran were found to be 62.31, 63.7 and 63.18, respectively. Bran treated with 50% ethanol had the highest L* and the value was significantly different to others. Both a* and b* values were higher for water washed bran than ethanol treated bran. Thus, it can be said that both 50% and 100% ethanol had a significant effect on all the three parameters. This suggested that the ethanol had positive impact on the bran color. Ethanol at strength 50% and 100% was found to be good for the removal of red pigments from the wheat bran as represented by lower a* values. Further

100% ethanol was found to be more effective for the removal of yellow pigments (b*). Similar results were found in the study by Saunders, et al. (2013), where ethanol was used to extract pigments from corn distillers dried grains with soluble (DDGS). Ethanol used as a solvent increased the brightness (Hunter L value) and decreased redness (Hunter a value) but there was no significant effect on b values (yellowness) in Saunders, et al. (2013) study.

Ethanol at 50% was found to be as efficient as ethanol at 100% concentration in removing the color pigments. Ethanol can dissolve both polar and non-polar substances because of its solubility properties. Thus, it is easily soluble in water in all proportions (Delgado & Martínez, 2013). The polarity index of water, 50% ethanol and 100% ethanol are 9, 7.1 and 5.2, respectively. The polarity index increases with polarity (Tang, et al., 2005). Polarity is highly related with solubility since polar molecules are soluble in polar solvent while non-polar molecules are soluble in non-polar solvent. 50% ethanol solution has ethanol and water in equal proportion. When wheat bran was soaked in 50% ethanol, bran absorbed water and it was swollen completely thereby increasing the surface area. That is why 50% ethanol might have been effective in removing the pigments. Further, fat content of flour incorporated with 50% ethanol treated bran was found to be significantly lower, suggesting better fat removal and color pigments with 50% ethanol.

Table 15. Overall color comparison of wheat bran from five HRSW and the bran obtained after treatment with water and ethanol (50% and 100%).

| Factors | Bran | L* Mean | a* Mean | b* Mean |
|---|---|---|---|---|
| Cultivars | Advance | 61.35e | 6.1b | 16.54e |
| | Prevail | 64.01b | 5.97c | 17.31a |
| | Select | 63.49c | 6.00bc | 16.92c |
| | Brick | 61.91d | 6.22a | 16.72d |
| | Forefront | 64.57a | 5.85d | 17.04a |
| Treatments | Water | 62.31c | 6.37a | 17.07a |
| | Ethanol 50% | 63.7a | 5.83b | 16.95b |
| | Ethanol 100% | 63.18b | 5.88b | 16.69c |

Values followed by the same letter in the same column are not significantly different ($p < 0.05$).
Water = wheat bran treated with water, ethanol 50% = wheat bran treated with 50% ethanol, and ethanol 100% = wheat bran treated with 100% ethanol.

Table 16. Mean and standard deviation (S.D.) of L*, a* and b* of water and ethanol treated wheat bran.

| Cultivars | Treatments | L* | | a* | | b* | | |
|---|---|---|---|---|---|---|---|---|
| | | Mean | S.D. | Mean | S.D. | Mean | S.D. | N |
| Advance | Ethanol 100% | 61.24 | 0.23 | 6.06 | 0.08 | 16.29 | 0.13 | 5 |
| | Ethanol 50% | 61.59 | 0.46 | 5.80 | 0.14 | 16.58 | 0.10 | 5 |
| | Water | 61.22 | 0.61 | 6.44 | 0.16 | 16.73 | 0.10 | 5 |
| Brick | Ethanol 100% | 61.78 | 0.50 | 6.06 | 0.11 | 16.43 | 0.14 | 5 |
| | Ethanol 50% | 61.97 | 0.24 | 5.88 | 0.12 | 16.42 | 0.11 | 5 |
| | Water | 61.98 | 0.19 | 6.73 | 0.12 | 17.29 | 0.11 | 5 |
| Forefront | Ethanol 100% | 64.85 | 0.20 | 5.86 | 0.06 | 17.17 | 0.19 | 5 |
| | Ethanol 50% | 64.99 | 0.11 | 5.69 | 0.06 | 16.94 | 0.20 | 5 |
| | Water | 63.86 | 0.17 | 5.97 | 0.08 | 17.07 | 0.09 | 5 |
| Prevail | Ethanol 100% | 63.37 | 0.31 | 5.74 | 0.07 | 16.78 | 0.20 | 5 |
| | Ethanol 50% | 65.84 | 0.33 | 5.84 | 0.38 | 17.73 | 0.27 | 5 |
| | Water | 62.83 | 0.37 | 6.34 | 0.20 | 17.41 | 0.13 | 5 |
| Select | Ethanol 100% | 64.67 | 0.46 | 5.71 | 0.13 | 16.82 | 0.15 | 5 |
| | Ethanol 50% | 64.12 | 0.43 | 5.92 | 0.16 | 17.05 | 0.15 | 5 |
| | Water | 61.66 | 0.47 | 6.37 | 0.12 | 16.88 | 0.17 | 15 |

Water = bran treated with water, ethanol 50% = bran treated with 50% ethanol, ethanol 100% = bran treated with 100% ethanol, and S.D. = standard deviation.

4.5.2. Whole wheat flour color

Comparison of whole wheat flour from different wheat varieties and the wheat flours incorporated with the treated bran are summarized in tables 17-18. Brightness of flour as expressed in terms of L* were in the range of 80.72-83.96. The highest L* value was obtained for Forefront flour followed by Brick flour and the lowest was for Prevail flour as shown in table 17. There was no significant differences in L* value among Advance, Select and Brick flours. In terms of redness (a*) and yellowness (b*), Prevail flour had the highest value. This means Prevail flour was darker in color in terms of all the three parameters, L*, a* and b*. Brick flour had the lowest a* and b* value and this was followed by Forefront and Select.

The flour incorporated with 50% ethanol treated bran had the highest L* value and the lowest a* and b* value as shown in table 17. There was no significant difference between WWF and flour incorporated with water treated bran in terms of L* value. Highest a* (redness) was obtained for the water treated bran and the highest b* (yellowness) was obtained for the control (WWF). As expected, Forefront flour had the highest L* since forefront bran was brightest of all.

Table 17. Comparison of color among whole wheat flours and among flours incorporated with treated bran.

| Factors | L* | a* | b* |
|---|---|---|---|
| Cultivars | | | |
| Advance | 82.52b | 1.44b | 10.52b |
| Prevail | 81.69c | 1.63a | 10.78a |
| Select | 82.68b | 1.39b | 10.22c |
| Brick | 82.38b | 1.29c | 10.20c |
| Forefront | 83.18a | 1.45b | 10.36bc |
| Treatments | | | |
| Control | 81.95c | 1.38b | 10.82a |
| (WWF) | 82.08c | 1.71a | 10.64b |
| Water | 83.18a | 1.28c | 10.11c |
| Ethanol 50% | 82.76b | 1.38b | 10.11c |
| Ethanol 100% | | | |

Values followed by the same letter in the same column are not significantly different ($p<0.05$).
Control (WWF) = whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, and ethanol 100% = flour incorporated with bran treated with 100% ethanol.

Table 18. Mean and standard deviation of L*, a* and b* among whole wheat flours from different cultivars and flour incorporated with treated brans.

| Cultivars | Treatments | L* Mean | S.D. | a* Mean | S.D. | b* Mean | S.D. | N |
|---|---|---|---|---|---|---|---|---|
| Advance | Ethanol 100% | 82.01 | 0.38 | 1.44 | 0.05 | 9.91 | 0.2 | 5 |
| | Ethanol 50% | 82.48 | 0.24 | 1.3 | 0.09 | 10.3 | 0.22 | 5 |
| | Water | 81.93 | 0.35 | 1.58 | 0.16 | 10.87 | 0.41 | 5 |
| | Control (WWF) | 83.67 | 0.39 | 1.43 | 0.07 | 10.99 | 0.18 | 5 |
| Brick | Ethanol 100% | 82.54 | 0.4 | 1.33 | 0.15 | 10.45 | 0.21 | 5 |
| | Ethanol 50% | 83.25 | 0.58 | 1.01 | 0.1 | 9.57 | 0.48 | 5 |
| | Water | 82.81 | 0.49 | 1.44 | 0.17 | 9.98 | 0.4 | 5 |
| | Control (WWF) | 80.93 | 0.4 | 1.37 | 0.11 | 10.81 | 0.24 | 5 |
| Forefront | Ethanol 100% | 83.48 | 0.62 | 1.38 | 0.2 | 10.26 | 0.44 | 5 |
| | Ethanol 50% | 83.96 | 0.75 | 1.22 | 0.13 | 9.76 | 0.19 | 5 |
| | Water | 82.85 | 0.36 | 1.76 | 0.08 | 10.59 | 0.15 | 5 |
| | Control (WWF) | 82.45 | 0.35 | 1.43 | 0.16 | 10.82 | 0.24 | 5 |
| Prevail | Ethanol 100% | 82.68 | 0.41 | 1.39 | 0.06 | 9.92 | 0.42 | 5 |
| | Ethanol 50% | 82.52 | 0.23 | 1.6 | 0.06 | 11.2 | 0.38 | 5 |
| | Water | 80.84 | 0.47 | 2.14 | 0.13 | 11.45 | 0.11 | 5 |
| | Control (WWF) | 80.72 | 0.43 | 1.37 | 0.12 | 10.57 | 0.5 | 5 |
| Select | Ethanol 100% | 83.07 | 0.45 | 1.35 | 0.13 | 10 | 0.35 | 5 |
| | Ethanol 50% | 83.69 | 0.29 | 1.28 | 0.11 | 9.66 | 0.14 | 5 |
| | Water | 81.97 | 0.63 | 1.63 | 0.14 | 10.32 | 0.17 | 5 |
| | Control (WWF) | 81.98 | 0.59 | 1.3 | 0.11 | 10.9 | 0.12 | 5 |

Control (WWF) = whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, ethanol 100% = flour incorporated with bran treated with 100% ethanol, and S.D. = standard deviation.

4.6. Gluten content

One way of determining the wheat quality and quantity is the determination of total gluten and the Gluten Index (GI). The dry gluten content of Advance, Prevail, Select, Brick, and Forefront flour were found to be 11.64, 11.66, 12.98, 10.89, and 9.88, respectively. Select flour had the highest and Forefront had the lowest dry gluten content and their dry gluten content was significantly different from other samples. There was no significant difference among Advance flour and Prevail flour in terms of dry and wet gluten content.

The percentage of wet gluten remaining on the sieve after washing with salt solution and centrifugation is the gluten index. GI of Select flour was significantly lower while GI of Brick, Advance and Forefront were significantly higher. In general a value of GI between 55-100 is considered to be suitable for bread making, but GI below 40 are appropriate for animal feed. Flour with GI greater than 80% is considered to be strong wheat (Barros, Alviola, et al., 2010b; Gil, et al., 2011; Oikonomou, et al., 2015) while GI below 50 are weaker (Barros, Alviola, et al., 2010b). GI value for all the five wheat varieties were found to be higher than 90%. Several studies have suggested hard red spring wheat (HRSW) to be superior to hard red winter wheat (HRWW) in regard to GI (E. Maghirang, et al., 2006). GI is a measure of gluten quality. Advance, Brick and Forefront were found to have the highest GI. Though gluten content was found to be higher for Select flour, its GI was the lowest. This suggested that the gluten quality of Select flour was not superior. Gluten quality is highly correlated with Farinograph properties; water absorption (WA), dough development time (DDT) and dough stability

(DS). As shown in table 20, Select flour had the lowest DDT and DS. Inferior gluten quality may be the reason behind it.

Table 19. Gluten content of five varieties of wheat flour.

| Wheat cultivars | Wet gluten% | Dry gluten% | GI (%) |
|---|---|---|---|
| Advance | 31.55±1.93$^{c}$ | 11.12±0.88$^{bc}$ | 98.75±0.94$^{a}$ |
| Prevail | 34.47±0.5$^{b}$ | 11.71±0.25$^{b}$ | 95.54±1.66$^{b}$ |
| Select | 37.14±1.92$^{a}$ | 12.91±0.25$^{a}$ | 90.28±3.45$^{c}$ |
| Brick | 29.50±0.63$^{d}$ | 10.76±0.42$^{c}$ | 99.03±0.92$^{a}$ |
| Forefront | 29.57±1.84$^{d}$ | 10.08±0.57$^{d}$ | 96.5±2.24$^{ab}$ |

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

## 4.7. Rheological properties of dough

Gluten, the major protein present in wheat flour, is composed of glutenin and gliadin. The glutenin complex provides the major viscoelastic properties to wheat flour dough. Gliadin protein is not as cohesive as glutenin protein and they give the dough its extensibility and viscosity. There are two chemical bonds in the dough system. They are covalent and non-covalent bonds. The two important covalent bonds are peptide bonds and disulphide bonds. Disulphide bonding of the polypeptide subunits of the glutenin complex is responsible for the viscoelastic properties of dough. Disulfide bonds are important for its cohesion. In glutenin, the disulfide bonds actually bind together protein chains with varying molecular weights ranging from 30,000 to 140,000 to form giant structures with higher molecular weights (HMW). Glutenin polymers are therefore, difficult to  dissolve in water due to their larger size (Khan & Shewry, 2009).

### 4.7.1. Brabender Farinograph analysis

Tables 20 and 21 provide Farinograph data on five varieties of whole wheat flour. In the current study, it was found that treating the bran with ethanol had beneficial impact on dough rheological properties. Water absorption increased and MTI level decreased in flour incorporated with bran treated with 50% ethanol in comparison to control (WWF). Five parameters were determined using the Brabender Farinograph. They were water absorption on 14% moisture basis (WA), dough development time (DDT), dough stability (DS), tolerance index (MTI) and breakdown time/departure time (BT).

Table 20. Comparison of farinograph parameters among ground whole wheat flours and flour incorporated with treated bran.

| | WA (%) | DDT (min) | Stability (min) | MTI (BU) | BT(min) |
|---|---|---|---|---|---|
| Cultivars | | | | | |
| Advance | 65.86bc | 7.33c | 11.64c | 21.25b | 15.14c |
| Prevail | 63.29d | 8.79b | 13.36b | 19.50b | 17.89ab |
| Select | 66.29b | 6.34c | 10.26d | 27.75a | 12.65d |
| Brick | 66.85a | 10.5a | 15.29a | 20.63b | 19.04a |
| Forefront | 65.66c | 7.56c | 13.95b | 18.13b | 16.8b |
| Treatments | | | | | |
| Control (WWF) | 63.71c | 6.44c | 7.86d | 28.4a | 11.99c |
| Water | 68.08a | 8.84ab | 12.02c | 23.4b | 16.17b |
| Ethanol 50% | 63.93c | 9.22a | 14.47b | 18.60c | 19.96a |
| Ethanol 100% | 66.64b | 7.91b | 17.25a | 15.4c | 17.09b |

Values followed by the same letter in same column are not significantly different ($p<0.05$).
Control (WWF) = whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, ethanol 100% = flour incorporated with bran treated with 100% ethanol, WA = water absorption (on 14% moisture basis), DDT = dough development time (minutes), stability = dough stability (minutes), MTI = mixing tolerance index (BU) and BT = breakdown time.

Table 21. Mean and standard deviation (S.D.) of overall Farinograph parameters of ground whole wheat flours and flour incorporated with the treated bran.

| Cultivars | Treatments | WA | S.D. | DDT | S.D. | Stability | S.D. | MTI | S.D. | BT | S.D. | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Advance | Ethanol 100% | 68.85 | 0.21 | 8.75 | 2.05 | 11.80 | 0.85 | 21.00 | 5.66 | 15.50 | 0.42 | 2 |
| | Ethanol 50% | 64.05 | 0.64 | 6.60 | 1.27 | 17.45 | 1.34 | 10.50 | 0.71 | 19.20 | 1.98 | 2 |
| | Water | 68.05 | 0.49 | 8.10 | 0.57 | 8.55 | 1.34 | 29.50 | 4.95 | 13.30 | 2.12 | 2 |
| | Control (WWF) | 62.50 | 0.00 | 5.85 | 0.21 | 8.75 | 1.63 | 24.00 | 5.66 | 12.55 | 1.77 | 2 |
| Prevail | Ethanol 100% | 67.10 | 0.14 | 8.50 | 0.71 | 11.65 | 0.07 | 23.00 | 4.24 | 15.10 | 0.14 | 2 |
| | Ethanol 50% | 60.85 | 0.21 | 11.20 | 0.28 | 16.75 | 0.35 | 13.50 | 2.12 | 24.20 | 1.13 | 2 |
| | Water | 65.35 | 0.35 | 9.25 | 1.06 | 16.90 | 2.26 | 15.50 | 3.54 | 20.30 | 2.26 | 2 |
| | Control (WWF) | 59.85 | 0.21 | 6.20 | 0.42 | 8.15 | 0.07 | 26.00 | 2.83 | 11.95 | 0.21 | 2 |
| Select | Ethanol 100% | 65.20 | 0.28 | 6.05 | 1.20 | 14.25 | 0.78 | 21.00 | 0.00 | 15.40 | 0.28 | 2 |
| | Ethanol 50% | 66.05 | 1.48 | 5.60 | 0.85 | 11.45 | 0.64 | 26.00 | 1.41 | 11.40 | 0.42 | 2 |
| | Water | 67.70 | 0.28 | 7.95 | 0.35 | 9.90 | 1.27 | 25.50 | 4.95 | 14.40 | 1.27 | 2 |
| | Control (WWF) | 66.20 | 0.57 | 5.75 | 0.07 | 5.45 | 0.35 | 38.50 | 0.71 | 9.40 | 0.14 | 2 |
| Brick | Ethanol 100% | 67.70 | 0.85 | 8.80 | 0.85 | 18.35 | 0.21 | 10.5 | 0.71 | 21.30 | 0.14 | 2 |
| | Ethanol 50% | 65.05 | 0.35 | 13.50 | 3.25 | 23.15 | 3.18 | 12.00 | 1.41 | 24.75 | 3.32 | 2 |
| | Water | 70.05 | 0.07 | 11.20 | 1.41 | 11.50 | 0.14 | 27.00 | 5.66 | 16.85 | 0.35 | 2 |
| | Control (WWF) | 64.60 | 0.14 | 8.50 | 0.00 | 8.15 | 0.35 | 33.00 | 8.49 | 13.25 | 1.48 | 2 |
| Forefront | Ethanol 100% | 64.35 | 0.07 | 7.45 | 1.06 | 16.30 | 0.00 | 17.50 | 4.95 | 18.15 | 1.48 | 2 |
| | Ethanol 50% | 63.65 | 0.35 | 9.20 | 0.00 | 17.45 | 0.35 | 15.00 | 0.00 | 20.25 | 0.35 | 2 |
| | Water | 69.25 | 0.07 | 7.70 | 0.00 | 13.25 | 0.92 | 19.50 | 6.36 | 16.00 | 0.71 | 2 |
| | Control (WWF) | 65.40 | 0.14 | 5.90 | 0.57 | 8.80 | 0.00 | 20.50 | 4.95 | 12.80 | 0.00 | 2 |

Control (WWF) = whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, ethanol 100% = flour incorporated with bran treated with 100% ethanol, WA = water absorption (on 14% moisture basis), DDT = dough development time (minutes), stability = Dough stability (minutes), MTI = mixing tolerance index (BU), BT = breakdown time, and S.D.= standard deviation.

Water absorption (14% moisture basis):

The mean value and standard deviation (S.D.) of water absorption (WA) for all the samples is listed in table 21. The average WA for Advance, Prevail, Select, Brick, and Forefront were found to be 65.86, 63.29, 66.29, 66.85, and 65.66%, respectively. Brick had significantly higher WA while Prevail had the lowest. In the case of treatments used, average water absorption value for flour incorporated with water treated bran, 50% ethanol treated bran and 100% ethanol treated bran were found to be 68.08, 63.93 and 66.64%. In a study by GUL and coworker (2009), water absorption (%) of wheat flour incorporated with 10% and 20% wheat bran was found to be 61.8% and 66.9%, respectively. Here, the increase of bran incorporation by 10% brought about 5% increase in Farinograph water absorption. This was because of increase of fiber content. Though flour was incorporated with 15% bran in our study, the water absorption was found to be in the range of GUL and coworker (2009) study for 20% wheat bran. This was because water absorption of flour is affected by other factors such as protein and starch content. Water treated bran had the highest water absorption (68.1%). This might be because flour incorporated with water washed bran had coarser particle size as explained by significantly higher amount of flour retention on the 40 mesh size sieve. There was no significant difference in the water absorption among control (WWF) and the flour containing 50% ethanol treated bran. Water absorption is one of the most important Farinograph parameters. This parameter determines the flour strength and is necessary for quality and price calculation of the finished product (Hadnađev, et al., 2011; Linlaud, et al., 2009). Water absorption is strongly related to the amount of damaged starch present, quality and quantity of gluten present and the fiber content. The amount of fiber present play a vital role in amount of water absorbed by the flour. Whole wheat flour has 12-15% fiber while refined white flours have below 2% fiber (Linlaud, et al., 2009). Water absorption varies with the amount of protein, starch and fiber. If the water absorption is higher than the optimal value, the dough becomes slurry and if it is low, the dough becomes powdery (Zaidel, et al., 2010). Researches have reported increased water absorption with incorporation of bran in flour (Noort, et al., 2010; D Zhang & Moore, 1999). This is because of different hydration properties of wheat bran which is further responsible for adverse effect on bread production (Noort, et al., 2010; Pomeranz, 1988b). In our study however, there was no difference in fiber content among the cultivars used in this study. Roozendaal, et al. (2012) reported adverse effect of bran incorporation when bran % used was equal to or above 20%. They claimed that though bran competes with starch and gluten for water during bread making, its affinity for water is low. They further explained that bran loses water under stress like high temperature causing disruption of gluten network and starch gelatinization. So, in our study bran incorporation was limited to 15%.

Particle size of the wheat bran might also affect water absorption. But in the study by Noort, et al. (2010), no significant effect of particle size on water absorption was observed. Jacobs, et al. (2015) studied the hydration properties of wheat bran. They found that water retention capacity was found to be affected but the Farinograph absorption was not affected by particle size. However, in another study by Sanz Penella, et al. (2008), water absorption was found to increase by 5% with increase in percentage of bran addition and lower bran particle size.

Dough development time (DDT):

Dough development is characterized by the complete hydration of protein and starch. This parameter is important for bakers to develop an optimally mixed dough. Overall DDT value varied from 5.6 to 13.5 minutes as shown in table 21. Since Brick cultivar had significantly high water absorption, it resulted in high DDT. There was no significant difference among DDT of Select, Advance and Forefront cultivar. In the case of bran treatment used, ethanol at 50% strength had the highest DDT while control (WWF) had the lowest. Water treatment and 100% ethanol treatment were not significantly different in terms of DDT. When wheat bran was treated with 50% ethanol, some fat might have been selectively removed which positively influence the rheological properties. So, flour incorporated with bran treated with ethanol at 50% strength might yield a higher DDT. DDT also depends upon the amount of gluten and fiber present like WA. In Barak, et al. (2013) study, DDT and dough stability increased in accordance to protein content of wheat varieties. Comparatively, Brick cultivar had the highest gluten index. Higher gluten index is related to higher gluten quality. This was the reason why Brick flour had the higher DDT value. Similarly, Select flour had the lowest gluten index and its DDT value was also lower. It also depends upon the particle size of the bran. According to Sanz Penella, et al. (2008), DDT increased with percentage of bran present and decreased with the particle size of the bran. However, in this study, though Prevail had coarse particle size, there was no effect on water absorption and DDT.

Mixing tolerance index (MTI):

MTI for five different wheat cultivars ranged between18.13-27.75 BU, as shown in table 21. The highest MTI was observed for Select wheat flour (27.75 BU). In the case of the treatments used, flour incorporated with bran treated with 50% ethanol and 100% ethanol had the lowest MTI with the values 18.6 and 15.4 BU, respectively. Flour with lower MTI is preferred in baking. In the study by Sanz Penella, et al. (2008), at higher bran concentration MTI was found to be decreased by 8% but with decreased bran particle size, MTI was found to be increased by 66%. In this study, % bran incorporation was same but there were differences in particle size. For flour incorporated with 50% and 100% ethanol treated bran, more flour was found to be retained in 200 and less than 200 mesh size sieve,. This means those flours had more fine bran particles. This might be the reason behind low MTI.

Dough stability:

Since Brick flour had significantly higher WA and DDT, its dough stability was also found to be higher. A longer mixing time results from strong flour because of the dense particles of stronger flour and slower water penetration (Zaidel, et al., 2010). Lower dough stability from flour with smaller bran particle fraction was observed in the Noort, et al. (2010) study indicating lower gluten network formation. Brick cultivar had the highest stability (15.29 minutes) and Select had the lowest (10.26 minutes) dough stability. Ethanol 100% incorporated flour had the highest stability (17.25 minutes) followed by ethanol 50% incorporated bran (14.47 minutes).

Breakdown time (BT):

Prevail and Brick wheat cultivars had the highest breakdown time with the values of 17.89 and 19.04 minutes, respectively. Select cultivar had the lowest BT with the value of 12.65 minutes. In terms of bran treatments used, control (WWF) had significantly low

BT while flour incorporated with 50% ethanol treated bran had the highest BT. There was no significant difference among flour incorporated with water and ethanol 100%.

Overall, Brick flour was found to be superior among other cultivars in terms of Farinograph parameters including water absorption (WA), dough development time (DDT), dough stability (DS), mixing tolerance index (MTI), and breakdown time (BT). In terms of gluten, though Brick flour had the lowest dry gluten content, gluten index value (GI) was the highest for Brick flour. GI is an important parameter used for the determination of gluten strength (Edwards, et al., 2007). It is an indication of whether the wheat is weak, normal or strong (E. B. Maghirang, et al., 2006). Similarly, high water absorption and high dough stability are the feature of strong flour (Migliori & Correra, 2013). This means among the five HRSW, Brick cultivar had highest dough strength.

In case of bran treatments used, ethanol 50% had overall higher DDT, higher stability, lower MTI and higher BT. Water absorption though was not found to be higher for the flour incorporated with 50% ethanol treated bran, it was difficult to relate how flour incorporated with ethanol treated bran affected every parameter. But since ethanol is a good solvent, it might have selectively removed fat from wheat bran that are responsible for undesirable dough rheological properties. Also, treated bran might have strengthened the protein matrix.

Increased DDT and decreased MTI with the increase in fiber content have been found in H and Prakash (2002) study. Higher water is absorbed by stronger flours than weaker flours because of higher protein content and dense particles of stronger flour (Zaidel, et al., 2010). Protein-protein interaction (Lazaridou, et al., 2007) and formation of three dimensional viscoelastic properties and gas retaining is the basic of dough development (Sliwinski, et al., 2004). Sanz Penella, et al. (2008) suggested negative correlationship of dough stability with MTI. Similar results were obtained in our study, as shown in the table 22. From the Pearson correlation coefficient, dough stability was found to be positively correlated to DDT ($r=0.592$) and BT ($r=0.926$) while negatively correlated to MTI ($r=-0.836$). Also the correlation was significant at 0.01 level ($p<0.01$). Dough stability was also found to be related to GI ($r=0.338$). MTI was again negatively correlated to GI ($r=-0.383$) and BT ($r=-0.806$).

Table 22. Pearson's correlation among Farinograph parameters.

| | WA | DDT | DS | MTI | BT | GI |
|---|---|---|---|---|---|---|
| WA | 1 | 0.139 | -0.099 | 0.178 | -0.13 | -0.103 |
| DDT | | 1 | 0.592** | -0.292 | 0.733** | 0.346 |
| DS | | | 1 | -0.836** | 0.926** | 0.338 |
| MTI | | | | 1 | -0.806** | -0.383* |
| BT | | | | | 1 | 0.383* |

WA=water absorption (14% moisture basis), DDT=dough development time (minutes), DS=dough stability (minutes), MTI=mixing tolerance index (BU), BT= breakdown time (minutes), and GI = gluten index (%)
**, * Correlation is significant at the 0.01and 0.05 level (2-tailed), respectively.

4.7.2. Dough extensibility test

This test was performed using Kieffer dough and gluten extensibility rig (A/KIE) using 5kg load cell. Table 24 and 25 provides the data for dough extensibility of whole wheat flour. The five parameters determined were resistance to extension or dough strength ($R_{max}$), maximum extensibility of dough ($E_{max}$), extensibility at $R_{max}$ ($E_{rmax}$), initial slope of the curve ($E_i$) and area.

Resistance to extension ($R_{max}$) and dough extensibility ($E_{max}$):

$R_{max}$ is the force required to pull the dough strip apart. Among the five different wheat cultivars, Brick and Prevail had significantly highest $R_{max}$, followed by Select cultivar, while Forefront had significantly lower $R_{max}$. In case of bran treatments used, flour incorporated with 50% ethanol treated bran had the maximum resistance to extension followed by flour with 100% ethanol treated bran. Control (WWF) had the lowest $R_{max}$. This suggested that the dough made from the flour incorporated with ethanol treated bran increased the dough strength.

Comparatively, control (WWF) had significantly high extensibility. Ethanol 50% and ethanol 100% had significantly low $E_{max}$ values. Similarly, among the wheat cultivars, Advance had the highest $E_{max}$ and Prevail had the lowest $E_{max}$ values. There was no significant differences among Select, Brick and Forefront in terms of $E_{max}$. Maximum dough extensibility ($E_{max}$) was found to be negatively correlated to dough resistance ($R_{max}$) (r=-0.641) and with Farinograph mixing tolerance (r=-0.267). Similar pattern of correlations were observed in previous research by Nash, et al. (2006).

WWF has total dietary fiber ranging from 11.5-15.5%. The incorporation of fiber affects the rheological properties of dough by disrupting the gas cells followed by breakdown of the three dimensional gluten network. This makes solid like property dough which in turn is responsible for reducing dough extensibility (Ahmed & Thomas, 2015). This was the reason why flour incorporated with ethanol treated bran had reduced extensibility ($E_{max}$) but higher dough strength ($R_{max}$). In the study by Ahmed and Thomas (2015), when some part of the flour was replaced with β-glucan, water absorption capacity increased but dough extensibility decreased. The same results were obtained in our study. Dough strength and dough extensibility are also the important factors in breadmaking.

Area:

The area under the curve is proportional to the energy required to bring about rupture of the test piece along the predetermined path, so sometimes it is expressed by energy. This is associated with both a large resistance and a large extensibility. But in our study area was found to be positively correlated to $R_{max}$ (r=0.875) and negatively correlated to $E_{max}$ (r=-0.298). The area of the curve obtained from control (WWF) dough was the lowest while for flour incorporated with 50% ethanol treated bran was the highest. There was no significant difference among Select, Brick and Advance wheat cultivars while Forefront had significantly lower area under the curve.

Extensibility at maximum resistance ($E_{Rmax}$):

$E_{Rmax}$ value for Advance cultivar (22.75 mm) was found to be higher followed by Brick and Forefront cultivar (20.92 and 20.46 mm, respectively). Prevail and Select values had the lowest $E_{Rmax}$ (19.36 and 20.03 mm, respectively) with no significant differences. For the bran treatments used, ethanol at 50% strength, had significantly high area (1026.32 g.mm) under the curve, while control (WWF) had significantly low area (690.05 g.mm).

Initial slope ($E_i$):

Gradient of force-distance curve during the first second of the test is $E_i$. The initial slope of the curve ($E_i$) for Brick and Prevail cultivar was found to be significantly higher (1.14 and 1.18 g/mm, respectively) while Advance and Forefront had the lowest (0.88 and 0.94 g/mm, respectively) $E_i$. For the bran treatments used, ethanol at 100% strength had significantly high $E_i$, followed by ethanol at 50% strength, as shown in table 24. Control (WWF) had significantly low $E_i$ value. The initial slope of the curve $E_i$ was found to be strongly correlated to $R_{max}$ (r=0.971) while negatively correlated to $E_{max}$ (r=-0.704) as shown in table 23.

Difference between $E_{max}$ and $E_{Rmax}$ ($E_{diff}$):

$E_{diff}$ was higher for Advance and Select cultivar while significantly lower value were observed for Brick and Prevail cultivars. Similarly, in the case of bran treatments used, control (WWF) had the highest $E_{diff}$ while ethanol at 50% strength and ethanol at 100% strength had the lowest $E_{diff}$. Since $E_{diff}$ is the difference between $E_{max}$ and $E_{Rmax}$, it is actually a parameter to measure how much the dough could be stretched further from the point when cross-links start to break to the point when dough ruptures completely. It is often referred as ‘extensional delay’ (Anderssen, et al., 2004). $E_{diff}$ was found to be strongly correlated to $E_{max}$ (r=0.961) while negatively correlated to $R_{max}$ (r=-0.697) as shown in table 23.

According to Anderssen and coworkers (2004), $R_{max}$ and $E_{max}$ together were not sufficient to differentiate among different flour samples, instead the most relevant parameters for determining the baking performance were $R_{max}$, $E_{max}$ and $E_{diff}$ . They claimed $E_{diff}$ (extensibility of dough before it ruptures) to be an important measure of the molecular dynamics of the dough. They also stated $E_{diff}$ to be an indicative of what happens in the gas cell walls during the final stage of baking. Due to the larger size of glutenin, they can form continuous network and is responsible for dough strength (resistance to deformation) and elasticity of dough while gliadin on the other hand, it acts as a plasticizer of the glutenin polymeric system (Goesaert, et al., 2005). GI, the measure of gluten quality, was highest for Brick cultivar. GI was found to be positively correlated to $R_{max}$, $E_{Rmax}$ and area while negatively correlated to $E_{max}$ and $E_{diff}$.

Table 23. Pearson's correlations among protein, extensibility parameters and Farinograph parameters.

| | Area | $R_{max}$ | $E_{max}$ | $E_{Rmax}$ | $E_i$ | $E_{diff}$ | GI |
|---|---|---|---|---|---|---|---|
| Area | 1 | 0.875** | -0.298** | 0.340** | 0.780** | -0.418** | 0.523** |
| $R_{max}$ | | 1 | -0.641** | 0.045 | 0.971** | -0.697** | 0.583** |
| $E_{max}$ | | | 1 | 0.356** | -0.704** | 0.961** | -0.069 |
| $E_{Rmax}$ | | | | 1 | -0.160* | 0.085 | 0.463** |
| $E_i$ | | | | | 1 | -0.703** | .0305 |
| $E_{diff}$ | | | | | | 1 | -0.307 |
| WA | 0.17 | 0.006 | 0.217 | 0.234 | -0.185 | 0.127* | -0.103 |
| DDT | 0.279 | 0.342* | -0.17 | 0.035 | 0.333* | -0.212 | 0.346 |
| DS | 0.258 | 0.26 | -0.079 | -0.014 | 0.27 | -0.084 | 0.338 |
| MTI | -0.275 | -0.267 | 0.011 | -0.046 | -0.255 | -0.099 | -0.383* |
| BT | 0.29 | 0.333* | -0.145 | 0.005 | 0.343* | 0.178 | 0.383* |

Area=area under the curve (g.mm), $R_{max}$=dough strength (g), $E_{max}$= dough extensibility (mm), $E_{Rmax}$= dough extensibility at $R_{max}$ (mm), $E_i$= intial slope of extensibility curve (g/mm), $E_{diff}$ = $E_{max}$-$E_{Rmax}$ (mm), GI = gluten index (%),WA=water absorption (14% moisture basis), DDT=dough development time (minutes), DS=dough stability (minutes), MTI=mixing tolerance index (BU), and BT= breakdown time (minutes).
** and * level of significance of 0.01 and 0.05, respectively (two-tailed).

Table 24. Comparison of dough extensibility among different samples and the treatments.

| | Area (g.mm) | $R_{max}$ (g) | $E_{max}$ (mm) | $E_{Rmax}$ (mm) | $E_i$ (g/mm) | $E_{diff}$ (mm) |
|---|---|---|---|---|---|---|
| Cultivars | | | | | | |
| Advance | 876.58a | 24.96c | 51.47a | 22.75a | 0.88c | 28.722a |
| Prevail | 831.32b | 28.82ab | 42.29c | 19.36d | 1.18a | 22.94c |
| Select | 854.56ab | 27.85b | 46.47b | 20.03cd | 1.11b | 26.45ab |
| Brick | 870.42a | 29.03a | 44.27bc | 20.92b | 1.14ab | 23.35c |
| Forefront | 774.46c | 23.91d | 46.42b | 20.46b | 0.94c | 25.96b |
| Treatments | | | | | | |
| Control (WWF) | 690.05d | 15.59d | 57.59a | 21.41a | 0.49d | 36.18a |
| Water | 744.66b | 24.08c | 43.78b | 20.21b | 0.94c | 23.57b |
| Ethanol 50% | 1026.32a | 36.23a | 42.08bc | 20.73ab | 1.29b | 21.35c |
| Ethanol 100% | 904.84b | 31.75b | 41.29c | 20.46b | 1.49a | 20.84c |

Values followed by the same letter on same column are not significantly different ($p<0.05$).
Control (WWF) = whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, ethanol 100% =flour incorporated with bran treated with 100% ethanol, Area=area under the curve (g.mm), $R_{max}$=dough strength (g), $E_{max}$= dough extensibility (mm), $E_{Rmax}$= dough extensibility at $R_{max}$ (mm), $E_i$= intial slope of extensibility curve (g/mm), and $E_{diff}$ = $E_{max}$-$E_{Rmax}$ (mm).
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Table 25. Extensibility test of ground whole wheat flour and flour incorporated with the treated bran using Kieffer dough and gluten extensibility Rig.

| Cultivars | Treatments | Area (g.mm) | S.D. | $R_{max}$ (g) | S.D. | $E_{max}$ (mm) | S.D. | $E_{Rmax}$ (mm) | S.D. | $E_i$ (g/mm) | S.D. | $E_{diff}$ (mm) | S.D. | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Advance | Ethanol 50% | 1157.3 | 67.4 | 37.04 | 3.43 | 44.69 | 4.41 | 22.9 | 1.08 | 1.38 | 0.16 | 21.8 | 4.39 | 10 |
| | Ethanol 100% | 826.05 | 84.35 | 24.65 | 2.02 | 47.06 | 4.26 | 22.12 | 1.00 | 0.88 | 0.08 | 24.9 | 4.36 | 10 |
| | Water | 628.73 | 32.92 | 18.64 | 2.25 | 52.3 | 11.1 | 20.53 | 2.97 | 0.67 | 0.2 | 31.8 | 9.67 | 10 |
| | Control (WWF) | 894.22 | 67.17 | 19.53 | 1.23 | 61.84 | 7.03 | 25.45 | 4.27 | 0.58 | 0.15 | 36.4 | 5.54 | 10 |
| Prevail | Ethanol 50% | 1220.8 | 87.78 | 46.8 | 2.46 | 39.8 | 4.9 | 21.37 | 1.69 | 1.95 | 0.21 | 18.4 | 5.14 | 10 |
| | Ethanol 100% | 689.85 | 35.39 | 24.95 | 1.31 | 39.61 | 1.19 | 18.03 | 0.94 | 1.09 | 0.11 | 21.6 | 1.47 | 10 |
| | Water | 825.98 | 72.88 | 28 | 0.91 | 40.65 | 4.01 | 20.18 | 1.45 | 1.12 | 0.08 | 20.5 | 3.48 | 10 |
| | Control (WWF) | 588.59 | 59.17 | 15.51 | 1.13 | 49.15 | 5.85 | 17.84 | 2.16 | 0.58 | 0.11 | 31.3 | 5.78 | 10 |
| Select | Ethanol 50% | 982.95 | 72.86 | 36.92 | 4.0 | 37.49 | 3.61 | 20.4 | 1.24 | 1.55 | 0.25 | 17.1 | 3.86 | 10 |
| | Ethanol 100% | 1116.8 | 100.0 | 36.89 | 2.73 | 45.86 | 4.51 | 22.11 | 1.98 | 1.44 | 0.21 | 23.8 | 3.95 | 10 |
| | Water | 767.94 | 31.53 | 25.71 | 1.22 | 41.51 | 3.86 | 18.82 | 1.01 | 1.08 | 0.11 | 22.7 | 3.11 | 10 |
| | Control (WWF) | 550.54 | 62.13 | 11.88 | 0.85 | 61.01 | 10 | 18.77 | 1.41 | 0.35 | 0.05 | 42.2 | 10.1 | 10 |
| Brick | Ethanol 50% | 860.68 | 74.23 | 30.52 | 4.07 | 43.38 | 5.45 | 19.12 | 0.78 | 1.32 | 0.24 | 24.3 | 5.52 | 10 |
| | Ethanol 100% | 1088.1 | 56.78 | 43.59 | 2.98 | 35.23 | 2.46 | 21.62 | 0.99 | 1.77 | 0.14 | 13.6 | 2.88 | 10 |
| | Water | 727.9 | 49.97 | 24.10 | 1.79 | 40.78 | 3.85 | 20.43 | 1.52 | 0.92 | 0.11 | 20.3 | 3.36 | 10 |
| | Control (WWF) | 804.99 | 118.9 | 17.91 | 2.43 | 57.69 | 7.97 | 22.53 | 2.16 | 0.57 | 0.15 | 35.2 | 7.56 | 10 |
| Forefront | Ethanol 50% | 909.84 | 57.62 | 29.85 | 1.68 | 45.06 | 3.94 | 19.88 | 1.61 | 1.24 | 0.13 | 25.2 | 4.57 | 10 |
| | Ethanol 100% | 803.37 | 22.89 | 28.69 | 1.33 | 38.73 | 3.56 | 18.41 | 0.89 | 1.27 | 0.11 | 20.3 | 3.59 | 10 |
| | Water | 772.74 | 60.19 | 23.97 | 1.93 | 43.67 | 2.56 | 21.11 | 1.95 | 0.89 | 0.14 | 22.6 | 3.15 | 10 |
| | Control (WWF) | 611.89 | 50.99 | 13.13 | 1.05 | 58.23 | 3.76 | 22.44 | 2.82 | 0.36 | 0.08 | 35.8 | 3.55 | 10 |

Control (WWF) = whole wheat flour obtained from the direct milling of whole wheat, water=flour incorporated with bran treated with water, ethanol 50% = flour incorporated with bran treated with 50% ethanol, ethanol 100% = flour incorporated with bran treated with 100% ethanol, Area=area under the curve (g.mm), $R_{max}$=dough strength (g), $E_{max}$= dough extensibility (mm), $E_{Rmax}$= dough extensibility at $R_{max}$ (mm), $E_i$= initial slope of extensibility curve (g/mm), $E_{diff}$ = $E_{max}$-$E_{Rmax}$ (mm), and S.D.= standard deviation.


Figure 12. SMS Kieffer Dough and gluten extensibility rig.

## 4.8. One dimensional extensibility of tortilla

Table 26 provides the overall result of one dimensional extensibility of tortilla. This test was performed using Tortilla/Pastry burst rig attached to Texture Analyzer TA.XT.*plus*. The objective of this test was to study the textural properties of whole wheat tortilla. The three parameters determined from this test were deformation modulus (ratio of rupture force and distance taken at the linear region of the curve), rupture force (force required to rupture the tortilla) and the rupture distance (distance up to which the tortilla extends before breaking).

Rupture distance and rupture force:

Rupture force and the rupture distance was significantly lower for control (WWF). There were no significant differences among the three bran treatments in terms of both the rupture distance and the rupture force. A high force required to rupture the tortilla indicates higher stretchability of tortilla. Similarly, stretchability of tortilla increases with rupture distance (Mao & Flores, 2001; L. Wang & Flores, 1999). This suggested that the tortilla made from flour incorporated with the treated bran had higher stretchability than control (WWF). The flour incorporated with bran treated with 50% and 100% ethanol, had the higher dough strength as represented by the higher $R_{max}$ value. So, tortilla made from flour incorporated with ethanol treated bran might have higher stretchability as represented by higher rupture distance and the rupture force.

Deformation modulus:

Elasticity is the ability of tortilla to return to its original form after the force is removed. In the case of tortilla, elasticity is expressed by Young’s modulus (also referred as deformation modulus) or gradient and is calculated as the slope of the initial straight line portion of the force in Newton kilograms. Thus, a lower gradient indicates more elasticity of tortilla (Prasopsunwattana, et al., 2009). Control (WWF) had significantly lower gradient. This suggested tortilla made from control (WWF) had significantly higher elasticity, compared to the treatments, water and ethanol.

Deformation modulus, rupture distance and maximum force all are related to tortilla extensibility or stretchability. Comparatively, greater force was needed to break the tortilla made from flour incorporated with the treated bran. This may be because of the higher dough strength ($R_{max}$) of flour incorporated with treated brans. The lower the deformation modulus, the higher the tortilla elasticity. Overall, treatments had significant effect on rupture distance and rupture force compared to control. This suggested that treated tortilla were good in relation to stretchability whereas control (WWF) was found to be good in terms of elasticity.

Table 26. One dimensional extensibility of tortilla.

| Treatments | Maximum force (N) | Rupture distance (mm) | Deformation modulus (N/mm) |
|---|---|---|---|
| Control (WWF) | 11.317±2.33[b] | 18.09±1.59[b] | 0.62±0.088[b] |
| Water | 14.49±0.55[a] | 20.33±0.57[a] | 0.71±0.045[a] |
| Ethanol 50% | 14.17±0.54[a] | 19.66±0.53[ab] | 0.72±0.009[a] |
| Ethanol 100% | 15.11±0.07[a] | 20.51±0.83[a] | 0.737±0.028[a] |

Values followed by the same letter in the same column are not significantly different ($p<0.05$).
Control (WWF) = tortilla made from the whole wheat flour obtained from the direct milling of whole wheat, Water=tortilla made from the flour incorporated with bran treated with water, ethanol 50% = tortilla made from the flour incorporated with bran treated with 50% ethanol, and ethanol 100% = tortilla made from the flour incorporated with bran treated with 100% ethanol.


Figure 13. Tortilla fitted on the Tortilla burst rig of the Texture Analyzer TA.XT.*plus*.

4.9. Color measurement of tortilla

There was no significant differences in whole wheat tortilla colors in terms of brightness (L*) among control (WWF) and tortilla made from the flour incorporated with the treated bran with water and 50% ethanol.

Yellowness value (b*) for the tortilla made from the control (WWF) and tortilla made from the flour incorporated with the treated bran with water and 50% ethanol, were higher (15.82, 15.16 and 15.22, respectively). Tortilla made from the flour incorporated with 100% ethanol treated bran was the lowest (13.09). Bran treated with 100% ethanol was found to have lower b* suggesting the removal of more yellow pigments from the bran. That was why tortilla made from flour incorporated with 100% ethanol treated bran had lower yellowness (b*). However, there was no significant differences among all the samples in terms of redness (a*). This suggested that only 100% ethanol had effect on tortilla colors in terms of yellowness (b*).

Table 27. Comparison of tortilla colors.

| Treatments | L* | a* | b* |
|---|---|---|---|
| Control (WWF) | 59.38a | 6.13a | 15.82a |
| Water | 60.15a | 5.34a | 15.16a |
| Ethanol 50% | 58.59ab | 5.74a | 15.22a |
| Ethanol 100% | 54.97b | 5.92a | 13.09b |

Values followed by the same letter in the same column are not significantly different ($p<0.05$).
Control (WWF) = tortilla made from the whole wheat flour obtained from the direct milling of whole wheat, Water=tortilla made from the flour incorporated with bran treated with water, ethanol 50% = tortilla made from the flour incorporated with bran treated with 50% ethanol, and ethanol 100% = tortilla made from the flour incorporated with bran treated with 100% ethanol.

4.10. Sensory analysis of whole wheat flour tortilla

The scoring scale for the sensory evaluation was ranged from 1 to 5 with 5 being the highest and 1 being the lowest score. Sensory analysis was performed in duplicate. Table 28 showed the overall sensory evaluation of tortilla. Participants found no difference among tortilla made from control (WWF) and tortilla made from the flour incorporated with the treated bran with water, 50% ethanol and 100% ethanol.

The means of treatments had no effect on sensory attributes and they were similar to control (WWF). This suggested that though ethanol had significant effect on dough rheological properties and tortilla stretchability, the sensory attributes of tortilla were not changed and were similar to the tortilla made from control (WWF).

Factors responsible for flavor and texture formation helps to improve the sensory attributes of cereal products containing whole grain or bran. White wheat flour provides less flavor to bread than whole grain wheat flour. This is due to the higher amount of volatile compounds and amino acids present in WWF (Heiniö, et al., 2016). In D Zhang and Moore (1999) study, soft white wheat bran bread was more preferred by test panelists over hard red spring wheat bran bread. White flour are lighter in color than whole grain products which may not be appealing for consumers.

Table 28. Mean sensory evaluation scores of Prevail whole wheat tortilla.

| Parameters | Control (WWF) | Water | Ethanol 50% | Ethanol 100% |
|---|---|---|---|---|
| Appearance | 4.16a | 3.75a | 4.00a | 3.83a |
| Aroma | 3.58a | 3.66a | 3.66a | 3.83a |
| Taste | 4.00a | 3.75a | 3.83a | 4.00a |
| Texture | 3.66a | 3.42a | 3.92a | 4.08a |
| Overall | 3.92a | 3.58a | 3.83a | 4.08a |

Values followed by the same letter in the same row are not significantly different ($p<0.05$).
Control (WWF) = tortilla made from the whole wheat flour obtained from the direct milling of whole wheat, Water=tortilla made from the flour incorporated with bran treated with water, ethanol 50% = tortilla made from the flour incorporated with bran treated with 50% ethanol, and ethanol 100% = tortilla made from the flour incorporated with bran treated with 100% ethanol.

Figure 14. Sensory analysis of tortilla.


Figure 15. Wheat bran treatment with 100% ethanol, 50% ethanol and water from left.


Figure 16. Tortilla making using Dough-Pro.

Figure 17. Tortilla cooking in a pan at $150^0$C.

# CHAPTER 5 CONCLUSION AND FUTURE WORK

## 5.1. Conclusions

The overall objective of this study was to understand the rheological and sensory changes of the flour incorporated with bran that was treated with water, ethanol at 50% and ethanol at 100% concentration. Wheat bran has been processed into ethanol using fermentative yeasts (Favaro, et al., 2012) but its treatment with solvent is limited. Ethanol is selective in removing pigments, oils, proteins, and other color producing constituents in agricultural materials. This research was done to determine whether bran treatment with ethanol will bring about desirable changes in the color, dough rheological properties and sensory characteristics of 100% whole wheat flour.

Nowadays wheat has been fortified with various other grains like soybean, rye and oat to increase its nutritional benefits. Studies on fortification of bread with hulls and cotyledon fibers isolated from peas, lentils and chickpeas has also been done (Dalgetty & Baik, 2006). Wheat is the richest source of dietary fiber, protein and carbohydrates. However, whole wheat products are dark in color, have undesirable flavor and show reduced dough rheological properties owing to the inclusion of bran. This research we attempted to improve the color, flavor and rheological properties of whole wheat products by modifying the wheat bran. Whole wheat tortilla was developed and evaluated using accepted quality evaluation tests.

In this study, five different HRSW cultivars (Advance, Prevail, Select, Brick and Forefront) from South Dakota were collected. They were tempered and milled. White flour and bran were separated by milling. White flour was stored in the refrigerator until use. Wheat bran was ground to fine particles using Restch mill and washed with water, 50% ethanol and 100% ethanol. After that, the treated bran was dried using a freeze dryer. The dried bran was reintroduced into the respective patent flour (15%) to make 100% WWF. Color measurement using Minolta spectrophotometer, proximate analysis, Farinograph analysis and dough extensibility were carried out. Gluten quality in terms of GI was also measured on patent white flour from the five wheat cultivars using Glutomatic system. Flour from one cultivar was used to make tortilla. Color measurement, one dimensional extensibility and sensory analysis were performed on the finished food product, namely, tortilla.

Bran washed with 50% ethanol had significantly higher lightness (L*) and lower redness (a*). Brick and Advance flour had significantly higher GI of 99.03% and 98.75%, respectively. DDT was significantly positively correlated to DS (r=0.592) and BT (r=0.733) at p<0.01. DDT and DS were also related to GI with r value of 0.346 and 0.338, respectively. MTI was negatively correlated to DS (r=-0.836), BT (r=-0.806) and GI (r=-0.383). Among the five cultivars, Brick was found to be superior in Farinograph data in relation to DDT, DS, BT, and MTI. Of the four bran treatments used, washing the wheat bran with 50% ethanol resulted in higher DDT, BT, $R_{max}$, $E_{Rmax}$, and lower MTI compared to the control (WWF). $R_{max}$ was negatively correlated to $E_{max}$ (-0.641) and $E_{diff}$ (r=-0.697) while positively correlated to area under the curve (r=0.875), Ei (r= 0.971) and GI (r= 0.83). Similarly, area and $E_i$ were negatively correlated to $E_{max}$ with r value of -0.298 and -0.704, respectively. Cultivars Brick and Prevail had significantly higher $R_{max}/E_i$ while cultivars Advance and Forefront had the lowest. WWF had significantly higher $E_{max}$ and lower area under the curve. The flour incorporated with 50% and 100%

ethanol treated bran had significantly lower $E_{max}$. Area under the curve was high only for flour with 50% ethanol treated bran. $E_{Rmax}$ for Advance cultivar was significantly high while Prevail/Select had significantly lower $E_{Rmax}$. Overall, Brick and Prevail cultivars were superior in terms of dough extensibility. Results from the one dimensional extensibility of tortilla experiment indicated that the tortilla made from the incorporation of treated bran with ethanol at 50% and 100% strength was good in terms of stretchability since they had significantly high rupture force and rupture distance, in compared to the control. However, control (WWF) was good only in the term of elasticity as expressed by low deformation modulus ($E_i$). No significant effect of treatments on tortilla colors and sensory attributes were found.

Higher DDT, DS, BT, and lower MTI are the desirable characteristics for good quality dough. $R_{max}$ and $E_{max}$ are inversely related. Greater $R_{max}$ determines the dough strength and $E_{max}$ determines the overall extensibility of the dough. Both of these are also the desirable characteristics of the dough for baking. A high force required to rupture the tortilla indicates higher stretchability of tortilla. Stretchability of tortilla increases with the rupture distance and the rupture force whereas, the elasticity increases with low deformation modulus.

The overall experiment was a completely randomized design with two factors (cultivar and treatments). Though bran treatment with ethanol did not have any significant effect on sensory attributes and color of tortilla, it had significant effect on Farinograph parameters and dough extensibility. A 50% ethanol solution was found to be more effective in selectively removing fat than a 100% ethanol solution. The polarity index of water, 50% ethanol, and 100% ethanol is 9, 7.1 and 5.2, respectively. The polarity index increases with polarity (Tang, et al., 2005). Polarity is highly related with solubility since polar molecules are soluble in polar solvent while non-polar molecules are soluble in non-polar solvent. A 50% ethanol solution has ethanol and water in equal proportion. When wheat bran was soaked in 50% ethanol solution, bran absorbed water and it was swollen completely thereby increasing the surface area. But in the case of 100% ethanol solution, bran could not be readily hydrated. This is why, ethanol at 50% strength might have been more effective in removing the color pigments and fat than ethanol at 100% strength.

## 5.2. Future work and recommendations

The purpose of the project was to improve the dough rheology by bran treatment with ethanol. Here ethanol and water treatment was compared to control (whole wheat flour). It would be interesting to compare ethanol treatment with more advance technique like supercritical fluid extraction (SFE).

The ethanol treatment had effect on Farinograph parameters. But the study on what was exactly removed by ethanol was not done. So, it would be interesting to know what was removed by ethanol 50%, ethanol 100% and water.

Hard red spring wheat (HRSW) from SD were used for the study. Differences among the cultivars were observed. HRSW would have been compared to hard red winter wheat (HRWW).Only sensory attributes of tortilla was studied. It would be good to study the storage stability of tortilla.

References

Adil, G. (2012). Whole-grain cereal bioactive compounds and their health benefits: a review. Journal of Food Processing & Technology.

Ahmed, J., & Thomas, L. (2015). Effect of β-D-glucan concentrate and water addition on extensional rheology of wheat flour dough. LWT - Food Science and Technology, 63(1), 633-639.

Albers, S., Muchová, Z., & Fikselová, M. (2009). The effects of different treated brans additions on bread quality. Scientia Agriculturae Bohemica, 40(2), 67-72.

Alviola, J. N., Jondiko, T. O., & Awika, J. M. (2012). Effect of Strong Gluten Flour on Quality of Wheat Tortillas Fortified with Cross-Linked Resistant Starch. Journal of Food Processing and Preservation, 36(1), 38-45.

Alviola, J. N., & Waniska, R. D. (2008). Determining the role of starch in flour tortilla staling using alpha-amylase. Cereal chemistry, 85(3), 391-396.

Anderson, J. W., Baird, P., Davis, R. H., Jr., Ferreri, S., Knudtson, M., Koraym, A., Waters, V., & Williams, C. L. (2009). Health benefits of dietary fiber. Nutr Rev, 67(4), 188-205.

Anderssen, R. S., Bekes, F., Gras, P. W., Nikolov, A., & Wood, J. T. (2004). Wheat-flour dough extensibility as a discriminator for wheat varieties. Journal of Cereal Science, 39(2), 195-203.

Andersson, A. A. M., Andersson, R., Piironen, V., Lampi, A.-M., Nyström, L., Boros, D., Fraś, A., Gebruers, K., Courtin, C. M., Delcour, J. A., Rakszegi, M., Bedo, Z., Ward, J. L., Shewry, P. R., & Åman, P. Contents of dietary fibre components and their relation to associated bioactive components in whole grain wheat samples from the healthgrain diversity screen. Food Chemistry, 136(3–4), 1243-1248.

Anton, A. (2008). Improving the nutritional and textural properties of wheat flour tortillas. Cereal Research Communications, 36(2), 301-311.

Auffret, A., Ralet, M., Guillon, F., Barry, J., & Thibault, J. (1994). Effect of grinding and experimental conditions on the measurement of hydration properties of dietary fibres. LWT-Food Science and Technology, 27(2), 166-172.

Balandrán-Quintana, R. R., Mercado-Ruiz, J. N., & Mendoza-Wilson, A. M. (2015a). Wheat Bran Proteins: A Review of Their Uses and Potential. Food Reviews International, 31(3), 279-293.

Balandrán-Quintana, R. R., Mercado-Ruiz, J. N., & Mendoza-Wilson, A. M. (2015b). Wheat bran proteins: a review of their uses and potential. Food Reviews International(just-accepted).

Barak, S., Mudgil, D., & Khatkar, B. S. (2013). Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. LWT - Food Science and Technology, 51(1), 211-217.

Barros, F., Alviola, J. N., & Rooney, L. W. (2010). Comparison of quality of refined and whole wheat tortillas. Journal of Cereal Science, 51(1), 50-56.

Barros, F., Alviola, J. N., Tilley, M., Chen, Y. R., Pierucci, V. R. M., & Rooney, L. W. (2010a). Predicting hot-press wheat tortilla quality using flour, dough and gluten properties. Journal of Cereal Science, 52(2), 288-294.

Barros, F., Alviola, J. N., Tilley, M., Chen, Y. R., Pierucci, V. R. M., & Rooney, L. W. (2010b). Predicting hot-press wheat tortilla quality using flour, dough and gluten properties. Journal of Cereal Science.

Bejosano, F. P., Joseph, S., Lopez, R. M., Kelekci, N. N., & Waniska, R. D. (2005). Rheological and sensory evaluation of wheat flour tortillas during storage. Cereal chemistry, 82(3), 256-263.

Bonnand-Ducasse, M., Della Valle, G., Lefebvre, J., & Saulnier, L. (2010). Effect of wheat dietary fibres on bread dough development and rheological properties. Journal of Cereal Science, 52(2), 200-206.

Butt, M. S., Nasir, M., Akhtar, S., & Sharif, K. (2004). Effect of moisture and packaging on the shelf life of wheat flour. Internet Journal of Food Safety, 4, 1-6.

Chen, H., Rubenthaler, G., Leung, H., & Baranowski, J. (1988). Chemical, physical, and baking properties of apple fiber compared with wheat and oat bran. Cereal Chem, 65(3), 244-247.

Chen, Y., Dunford, N. T., & Goad, C. (2013). Evaluation of wheat bran obtained by tangential abrasive dehulling device. Food and Bioprocess Technology, 6(7), 1655-1663.

Curtis, B. C., Rajaram, S., & Macpherson, H. G. (2002). Bread wheat: improvement and production.

Dalgetty, D. D., & Baik, B.-K. (2006). Fortification of bread with hulls and cotyledon fibers isolated from peas, lentils, and chickpeas. Cereal Chemistry, 83(3), 269-274.

Darly-Kindelspire, J. (2013). Multi-instrumental Approaches to Determine End-use Quality in Wheat. Health and Nutritional Sciences Department, South Dakota State University.

Delgado, D. R., & Martínez, F. (2013). Solution thermodynamics of sulfadiazine in some ethanol + water mixtures. Journal of Molecular Liquids, 187, 99-105.

Dobraszczyk, B., & Morgenstern, M. (2003). Rheology and the breadmaking process. Journal of Cereal Science, 38(3), 229-245.

Dunnewind, B., Sliwinski, E., Grolle, K., & Vliet, T. v. (2003). The kieffer dough and gluten extensibility rig-an experimental evaluation. Journal of Texture Studies, 34(5-6), 537-560.

Dunnewind, B., Sliwinski, E. L., Grolle, K. C. F., & Vliet, v. T. (2003). The Kieffer dough and gluten extensibility rig - An experimental evaluation. In,  (pp. 537).

Dykes, L., & Rooney, L. (2007). Phenolic compounds in cereal grains and their health benefits. Cereal Foods World, 52(3), 105-111.

Edwards, N. M., Gianibelli, M. C., McCaig, T. N., Clarke, J. M., Ames, N. P., Larroque, O. R., & Dexter, J. E. (2007). Relationships between dough strength, polymeric protein quantity and composition for diverse durum wheat genotypes. Journal of Cereal Science, 45(2), 140-149.

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutrition Research Reviews, 23(01), 65-134.

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev, 23(1), 65-134.

Favaro, L., Basaglia, M., & Casella, S. (2012). Processing wheat bran into ethanol using mild treatments and highly fermentative yeasts. Biomass and Bioenergy, 46, 605-617.

Fistes, A., Rakic, D., & Takaci, A. (2013). The function for estimating the separation efficiency of the wheat flour milling process. Journal of Food Science and Technology, 50(3), 609-614.

Garg, R., & Dahiya, S. (2003). Nutritional evaluation and shelf life studies of papads prepared from wheat–legume composite flours. Plant Foods for Human Nutrition, 58(4), 299-307.

Ge, Y., Sun, A., Ni, Y., & Cai, T. (2000). Some nutritional and functional properties of defatted wheat germ protein. Journal of Agricultural and Food Chemistry, 48(12), 6215-6218.

Gil, D. H., Bonfil, D., & Svoray, T. (2011). Multi scale analysis of the factors influencing wheat quality as determined by Gluten Index. Field Crops Research, 123(1), 1-9.

Gillies, S. A., Futardo, A., & Henry, R. J. (2012). Gene expression in the developing aleurone and starchy endosperm of wheat. Plant biotechnology journal, 10(6), 668-679.

Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K., & Delcour, J. A. (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. Trends in Food Science & Technology(1-3).

Gómez, M., Jiménez, S., Ruiz, E., & Oliete, B. (2011). Effect of extruded wheat bran on dough rheology and bread quality. LWT - Food Science and Technology, 44(10), 2231-2237.

Gómez, M., Ronda, F., Blanco, C., Caballero, P., & Apesteguía, A. (2003). Effect of dietary fibre on dough rheology and bread quality. European Food Research and Technology, 216(1), 51-56.

Grooms, K. N., Ommerborn, M. J., Pham, D. Q., Djoussé, L., & Clark, C. R. (2013). Dietary fiber intake and cardiometabolic risks among US Adults, NHANES 1999-2010. The American journal of medicine, 126(12), 1059-1067. e1054.

GÜL, H., ÖZer, M. S., & DİZlek, H. (2009). Improvement of the Wheat and Corn Bran Bread Quality by Using Glucose Oxidase and Hexose Oxidase. Journal of Food Quality, 32(2), 209-223.

Guo, G., Jackson, D. S., Graybosch, R. A., & Parkhurst, A. M. (2003). Wheat tortilla quality: impact of amylose content adjustments using waxy wheat flour.

H, A. S., & Prakash, J. (2002). Wheat bran (Triticum aestivum): composition, functionality and incorporation in unleavened bread. Journal of Food Quality, 25(3), 197.

Hadnađev, T. D., Torbica, A., Pojić, M., & Hadnađev, M. (2011). The role of empirical rheology in flour quality control: INTECH Open Access Publisher.

Heiniö, R. L., Noort, M. W. J., Katina, K., Alam, S. A., Sozer, N., de Kock, H. L., Hersleth, M., & Poutanen, K. (2016). Sensory characteristics of wholegrain and bran-rich cereal foods – A review. Trends in Food Science & Technology, 47, 25-38.

Helrich, K. (1990). Official methods of Analysis of the AOAC. Volume 2: Association of Official Analytical Chemists Inc.

Hlynka, I. (1964). Wheat chemistry and technology: St. Paul, Minn., American Association of Cereal Chemists, 1964.

Hoseney, R. C. (1994). Principles of cereal science and technology: American Association of Cereal Chemists (AACC).

Hu, C., Cai, Y.-Z., Li, W., Corke, H., & Kitts, D. D. (2007). Anthocyanin characterization and bioactivity assessment of a dark blue grained wheat (Triticum aestivum L. cv. Hedong Wumai) extract. Food Chemistry, 104(3), 955-961.

Jacobs, P. J., Hemdane, S., Dornez, E., Delcour, J. A., & Courtin, C. M. (2015). Study of hydration properties of wheat bran as a function of particle size. Food Chem, 179, 296-304.

Javed, M. M., Zahoor, S., Shafaat, S., Mehmooda, I., Gul, A., Rasheed, H., Bukhari, S. A. I., & Aftab, M. N. (2012). Wheat bran as a brown gold: Nutritious value and its biotechnological applications. African Journal of Microbiology Research, 6(4), 724-733.

Jensen, M. K., Koh-Banerjee, P., Hu, F. B., Franz, M., Sampson, L., Grønbæk, M., & Rimm, E. B. (2004). Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. The American journal of clinical nutrition, 80(6), 1492-1499.

Khan, K., & Shewry, P. R. (2009). Wheat: chemistry and technology: American Association of Cereal Chemists, Inc (AACC).

Krishnan, P., & Darly-Kindelspire, J. (2013). Food Product Innovations Using Soy Ingredients. Journal of Human Nutrition & Food Science.

Kumar, G. S., & Krishna, A. G. (2013). Studies on the nutraceuticals composition of wheat derived oils wheat bran oil and wheat germ oil. Journal of Food Science and Technology, 1-7.

Laurikainen, T., Härkönen, H., Autio, K., & Poutanen, K. (1998). Effects of enzymes in fibre-enriched baking. Journal of the Science of Food and Agriculture, 76(2), 239-249.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. Journal of Food Engineering, 79(3), 1033-1047.

Le Bleis, F., Chaunier, L., Chiron, H., Della Valle, G., & Saulnier, L. (2015). Rheological properties of wheat flour dough and French bread enriched with wheat bran. Journal of Cereal Science, 65, 167-174.

Lehtinen, O.-P. (2012). Modifying Wheat Bran for Food Applications-Effect of Wet Milling and Enzymatic Treatment.

Letang, C., Piau, M., & Verdier, C. (1999). Characterization of wheat flour–water doughs. Part I: Rheometry and microstructure. Journal of Food Engineering, 41(2), 121-132.

Linlaud, N., Puppo, M., & Ferrero, C. (2009). Effect of hydrocolloids on water absorption of wheat flour and farinograph and textural characteristics of dough. Cereal chemistry, 86(4), 376-382.

Liyana-Pathirana, C., & Shahidi, F. (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chemistry, 93(1), 47-56.

Maghirang, E., Lookhart, G., Bean, S., Pierce, R., Xie, F., Caley, M., Wilson, J., Seabourn, B., Ram, M., & Park, S. (2006). Comparison of quality characteristics and breadmaking functionality of hard red winter and hard red spring wheat. Cereal chemistry, 83(5), 520-528.

Maghirang, E. B., Lookhart, G. L., Bean, S. R., Pierce, R. O., Xie, F., Caley, M. S., Wilson, J. D., Seabourn, B. W., Ram, M. S., Park, S. H., Chung, O. K., & Dowell, F. E. (2006). Comparison of Quality Characteristics and Breadmaking Functionality of Hard Red Winter and Hard Red Spring Wheat. Cereal Chemistry, 83(5), 520-528.

Mann, G., Diffey, S., Allen, H., Pumpa, J., Nath, Z., Morell, M. K., Cullis, B., & Smith, A. (2008). Comparison of small-scale and large-scale mixing characteristics: Correlations between small-scale and large-scale mixing and extensional characteristics of wheat flour dough. Journal of Cereal Science, 47(1), 90-100.

Mao, Y., & Flores, R. A. (2001). Mechanical starch damage effects on wheat flour tortilla texture. Cereal Chemistry, 78(3), 286-293.

Meireles, M. A. A. (2008). Extracting bioactive compounds for food products: theory and applications: CRC press.

Migliori, M., & Correra, S. (2013). Modelling of dough formation process and structure evolution during farinograph test. International Journal of Food Science & Technology, 48(1), 121-127.

Müller-Lissner, S. A. (1988). Effect of wheat bran on weight of stool and gastrointestinal transit time: a meta analysis. British medical journal (Clinical research ed.), 296(6622), 615.

Nash, D., Lanning, S. P., Fox, P., Martin, J. M., Blake, N. K., Souza, E., Graybosch, R. A., Giroux, M. J., & Talbert, L. E. (2006). Relationship of dough extensibility to dough strength in a spring wheat cross. Cereal Chemistry(3).

Noort, M. W. J., van Haaster, D., Hemery, Y., Schols, H. A., & Hamer, R. J. (2010). The effect of particle size of wheat bran fractions on bread quality – Evidence for fibre–protein interactions. Journal of Cereal Science, 52(1), 59-64.

Oikonomou, N., Bakalis, S., Rahman, M., & Krokida, M. (2015). Gluten index for wheat products: main variables in affecting the value and nonlinear regression model. International Journal of Food Properties, 18(1), 1-11.

Oury, F. X., Leenhardt, F., Rémésy, C., Chanliaud, E., Duperrier, B., Balfourier, F., & Charmet, G. (2006). Genetic variability and stability of grain magnesium, zinc and iron concentrations in bread wheat. European Journal of Agronomy, 25(2), 177-185.

Özboy, Ö., & Köksel, H. (1997). Unexpected Strengthening Effects of a Coarse Wheat Bran on Dough Rheological Properties and Baking Quality. Journal of Cereal Science, 25(1), 77-82.

Pomeranz, Y. (1988a). Wheat : chemistry and technology. St. Paul: Minn., USA : American association of cereal chemists.

Pomeranz, Y. (1988b). Wheat: chemistry and technology: American Association of Cereal Chemists.

Prasopsunwattana, N., Omary, M. B., Arndt, E. A., Cooke, P. H., Flores, R. A., Yokoyama, W., Toma, A., Chongcham, S., & Lee, S. P. (2009). Particle Size

Effects on the Quality of Flour Tortillas Enriched with Whole Grain Waxy Barley. Cereal Chemistry(4).

Prückler, M., Siebenhandl-Ehn, S., Apprich, S., Höltinger, S., Haas, C., Schmid, E., & Kneifel, W. (2014). Wheat bran-based biorefinery 1: Composition of wheat bran and strategies of functionalization. LWT-Food Science and Technology, 56(2), 211-221.

Radenkovs, V., Klava, D., Krasnova, I., & Juhnevica-Radenkova, K. (2014). Application of enzymatic treatment to improve the concentration of bioactive compounds and antioxidant potential of wheat and rye bran. In 9th Baltic Conference on Food Science and Technology" Food for Consumer Well-Being" Foodbalt 2014, Jelgava, Latvia, 8-9 May, 2014, (pp. 127-132): Latvia University of Agriculture, Faculty of Food Technology.

Rasco, B., Borhan, M., Yegge, J., Lee, M., Siffring, K., & Bruinsma, B. (1991). Evaluation of enzyme and chemically treated wheat bran ingredients in yeast-raised breads. Cereal chemistry, 68(3), 295-299.

Reyes-Pérez, F., Salazar-García, M. G., Romero-Baranzini, A. L., Islas-Rubio, A. R., & Ramírez-Wong, B. (2013). Estimated glycemic index and dietary fiber content of cookies elaborated with extruded wheat bran. Plant foods for human nutrition, 68(1), 52-56.

Roozendaal, H., Abu-hardan, M., & Frazier, R. A. (2012). Thermogravimetric analysis of water release from wheat flour and wheat bran suspensions. Journal of Food Engineering, 111(4), 606-611.

Rot, C. R., & Rot, F. R. Southern Minnesota Wheat Research. Minnesota Wheat Research, 85.

Sakhare, S. D., & Inamdar, A. A. (2014). The cumulative ash curve: a best tool to evaluate complete mill performance. Journal of Food Science and Technology, 51(4), 795-799.

Sanz Penella, J. M., Collar, C., & Haros, M. (2008). Effect of wheat bran and enzyme addition on dough functional performance and phytic acid levels in bread. Journal of Cereal Science, 48(3), 715-721.

Saunders, J. A., Rosentrater, K. A., & Krishnan, P. G. (2013). Removal of Color Pigments From Corn Distillers Dried Grains With Solubles (DDGS) to Produce an Upgraded Food Ingredient. Journal of Food Research, 2(5), 111.

Schmiele, M., Jaekel, L. Z., Patricio, S. M. C., Steel, C. J., & Chang, Y. K. (2012). Rheological properties of wheat flour and quality characteristics of pan bread as modified by partial additions of wheat bran or whole grain wheat flour. International Journal of Food Science & Technology, 47(10), 2141-2150.

Serna-Saldivar, S. O., Guajardo-Flores, S., & Viesca-Rios, R. (2004). Potential of triticale as a substitute for wheat in flour tortilla production. Cereal chemistry, 81(2), 220-225.

Seyer, M.-È., & Gélinas, P. (2009). Bran characteristics and wheat performance in whole wheat bread. International Journal of Food Science & Technology, 44(4), 688-693.

Sliwinski, E. L., Kolster, P., Prins, A., & Vliet, T. v. (2004). On the relationship between gluten protein composition of wheat flours and large-deformation properties of their doughs. Journal of Cereal Science, 39(2), 247-264.

Sobota, A., Rzedzicki, Z., Zarzycki, P., & Kuzawińska, E. (2015). Application of common wheat bran for the industrial production of high-fibre pasta. International Journal of Food Science & Technology, 50(1), 111-119.

Soumya, D. (2011). Late stage complications of diabetes and insulin resistance. Journal of Diabetes & Metabolism.

Stevenson, L., Phillips, F., O'sullivan, K., & Walton, J. (2012). Wheat bran: its composition and benefits to health, a European perspective. International journal of food sciences and nutrition, 63(8), 1001-1013.

Sudha, M., Srivastava, A., & Leelavathi, K. (2007). Studies on pasting and structural characteristics of thermally treated wheat germ. European Food Research and Technology, 225(3-4), 351-357.

Tang, Q., Brown, J. H., & Fu, W. (2005). Simmondsin processing methods and products. In): Google Patents.

Tsen, C., Levi, I., & Hlynka, I. (1962). A rapid method for the extraction of lipids from wheat products. Cereal Chemistry, 39, 195-203.

Uslu, S., Kebapci, N., & Ozcelik, E. (2013). Metabolic syndrome and colorectal cancer: a review.

Van Hung, P., Maeda, T., & Morita, N. (2007). Dough and bread qualities of flours with whole waxy wheat flour substitution. Food Research International, 40(2), 273-279.

Wang, J., Sun, B., Cao, Y., Tian, Y., & Li, X. (2008). Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. Food Chemistry, 106(2), 804-810.

Wang, L., & Flores, R. A. (1999). Effect of different wheat classes and their flour milling streams on textural properties of flour tortillas 1. Cereal Chemistry, 76(4), 496-502.

Zaidel, A., Norulfairuz, D., Chin, N. L., & Yusof, Y. A. (2010). A review on rheological properties and measurements of dough and gluten. Journal of Applied Sciences, 10(20), 2478-2490.

Zhang, D., & Moore, W. R. (1997). Effect of wheat bran particle size on dough rheological properties. Journal of the Science of Food and Agriculture, 74(4), 490-496.

Zhang, D., & Moore, W. R. (1999). Wheat bran particle size effects on bread baking performance and quality. Journal of the Science of Food and Agriculture, 79(6), 805-809.

Zhao, F. J., Su, Y. H., Dunham, S. J., Rakszegi, M., Bedo, Z., McGrath, S. P., & Shewry, P. R. (2009). Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. Journal of Cereal Science, 49(2), 290-295.