Enhancing Antioxidant Activity and Extractability of Bioactive Compounds of Wheat Bran using Thermal Treatments

BY

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ABSTRACT

Wheat bran contains a diverse collection of macronutrients, micronutrients and bioactive components, including those thought to have an important role in reducing the risk of many chronic diseases. Wheat bran contains very significant levels of dietary fibre, phenolic compounds, phytate and lesser amounts of other micronutrients and phytochemicals such as B vitamins, minerals, lignans, sterols, and alkylresorcinols, all of which can provide health benefits beyond nutrition which is the basis for the characterization of wheat bran as a functional food. In particular, it is the antioxidant activity associated with many of the phytochemicals of wheat bran that has received considerable attention over the past decade with regard to functional properties and health benefits.

The complication of wheat bran as a functional food relates to its high insoluble fibre content, typically in the neighbourhood of 50%, which renders bran and its constituent bioactives largely indigestible, and hence with limited bioavailability until passage through the large intestine where the bran can be broken down by bacteria. Presumably, if the solubility of wheat bran could be enhanced by pre-treatment, its biological impact associated with its fibre and resident phytochemicals would also be enhanced. Among the treatments reported in the literature on wheat bran and analogous grain products, such as roasting, boiling, steaming, microwave heating and autoclaving, it is the latter that appears to provide some of the more interesting effects, although there have been very few studies. As well, because all digestive secretions are aqueous in nature, an underlying rationale for this thesis research is the use of aqueous extracts of wheat bran as the starting
point for analysis in order to provide a basis for future research focused on bioaccessibility and bioavailability.

The objectives of this thesis research were mainly three-fold: (1) evaluate the effects of bran autoclaving conditions compared to a few other thermal treatments such as air-oven and air-oven with N₂ on the antioxidant activity and phenolic content of water-soluble bran extracts, (2) examine in detail, the autoclaving process on antioxidant activity of water soluble extracts within the range of processing conditions available including sterilization time and temperature, and repeated treatment cycles, and (3) determine the effects of autoclaving on chemical composition of bran, water-soluble extracts and insoluble residues including composition of chemical constituents such as minerals and B vitamins, protein, starch, phytic acid, β-glucans, arabinoxylans, and dietary fiber.

Autoclaved wheat bran (using a standard cycle of 15 min sterilization and 10 min drying) was substantially improved in aroma that was obvious by subjective evaluation. Such bran appeared to be very stable, and even after several years at room temperature. In contrast, untreated bran typically revealed a rancid aroma after only one or two months. Autoclaving yielded bran with little to no enzyme activity as evaluated on the basis of polyphenol oxidase and peroxidase whose levels were considerable in untreated bran. This result may explain the stability of the wheat bran upon prolonged storage as presumably lipase and other enzymes that promote rancidity were not active. As well, water-soluble extracts from autoclaved bran, produced no effect on dough mixing, whereas water-soluble extracts of untreated bran resulted in the absence of dough formation. The presence of active xylanase in
extracts of untreated bran was likely responsible to destroy normal dough mixing properties by hydrolysis of arabinoxylans which have significant water binding properties disproportionate to their relatively low concentration in wheat endosperm and flour.

Freeze dried water soluble extracts of autoclaved bran had substantially enhanced levels of antioxidant activity compared to corresponding extracts from untreated bran. The level of enhancements in free radical scavenging activities were typically 300-400% in the many different experiments that were performed. Freeze dried water soluble extracts of autoclaved bran had significantly enhanced levels of phenolic content compared to that of untreated bran. However there was no close correspondence between AOA and total phenolic content. It was surmised that the Folin-Ciocalteu method used may not accurately estimate phenolic compounds and/or compounds with AOA extractable in water may not all be phenolic in nature, e.g. they could also be products of the presumed Maillard reaction.

Autoclave processing conditions in terms of number of autoclave cycles, sterilization time duration and temperature were very well characterized in terms of their effects on enhancing the antioxidant activity, as well as bran colour. Interestingly, the autoclaving effect to enhance antioxidant activity was largely manifested (~95% of enhanced antioxidant activity) after only one-third (i.e. 5 min) the duration of a standard autoclave cycle. In contrast, extending the autoclave cycle time or the number of autoclave cycles beyond the standard parameters resulted in increasingly negative sensory properties, i.e. bran became progressively darker and had a very noticeable burnt aroma. Results seemed in accord with formation of
Maillard reaction products. Additional evidence for Maillard reaction during autoclaving was substantially reduced content of amino acids and free sugars in autoclaved bran extracts. Results indicated that autoclaving bran at 121 °C for 10-15 min sterilization, plus a short drying cycle was close to ideal to obtain desired bran colour and aroma together with increased solubilisation of antioxidant compounds. Autoclaving of white wheat bran using these conditions produced a product with a very appealing golden colour.

Among the most interesting and compelling results of bran autoclaving was the observed enhancement in water extraction of a very broad spectrum of bran bioactive constituents including minerals, phytate, B vitamins, and non-starch polysaccharide fibre compounds suggesting enhanced bioaccessibility if not bioavailability of these phytochemicals. Compared to water soluble extracts of untreated bran, autoclaved bran produced extracts that were higher in mineral content (except phosphorus) by 45-85%. Phytate content was markedly enhanced due to autoclaving from a base level of ~ 2% in extracts of control bran to 10% in extracts of autoclaved dry. Phytic acid has traditionally been considered to be an antinutrient associated with its ability to bind divalent cations and reduce their bioavailability for people consuming very narrow diet of predominantly whole grains. However, antioxidant activity has also been ascribed to phytic acid which is known to have potent cancer preventive properties.

Except for thiamine, autoclave treatment of bran resulted in significant increases in extracted B vitamins, ranging from 22% (B9 folic acid) to 78% (B3 niacin) relative to corresponding extracts from untreated bran. For β-glucans which
were sparingly soluble in water extracts from untreated bran, levels in extracts of autoclaved bran increased to 2.7%. Likewise for arabinoxylan, extract concentration increased from 1.7% to 6.6% in untreated and autoclaved bran, respectively. Mirroring these results were similar outcomes for total dietary fibre and resistant oligosaccharides which increased in water soluble extracts of bran due to autoclaving, from 4% to 13.2%, and 8.8% to 22.7%, respectively. Autoclaving of dry bran was clearly shown to be extremely effective to enhance the fibre solubility of wheat bran.

The likely mechanism of these enhanced solubilities, which remains to be proven, is a combination of physical disruption of bran cell walls, thus enhancing fibre extractability, combined with hydrolysis of polysaccharides to smaller and more soluble components. As the phenolic compound, ferulic acid is a molecular component of arabinoxylans, the process also results, fortuitously, in enhanced levels of antioxidant activity in extracts, which could also be further enhanced by Maillard reaction products. Presumably the standard autoclave procedure combining high temperature, elevated pressure, an oxygen free atmosphere, and rapid depressurization towards the end of the cycle, all together contribute a unique and potent physicochemical effect to breakdown the structure of non-starch polysaccharide laden cell walls of bran, rendering constituent bioactive compounds much more readily extractable with water.

Results taken together were very compelling. They point to the development of novel wheat bran and extracts with considerable commercial potential for whole grain food use and a variety of nutraceutical applications. The autoclaving treatment that produces this novel bran represents a highly significant development in bran
processing technology that has the potential to add substantial value to common wheat bran for food and health applications far beyond what may be possible with whole grain wheat or normal bran in conventional foods. Perhaps most importantly, outcomes of the research described in this thesis point to the conclusion that the nutritional and functional food benefits of normal wheat bran probably represent a fraction of its potential due to the limited digestibility of wheat bran, which in turn, limits the bioavailability of its fiber and constituent bioactive compounds. Autoclaved bran appears to have no such limitation.
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1. INTRODUCTION

Consumption of whole wheat bread and other foods containing whole grain in general, has increased dramatically in the past decade. Between 2000 and 2006, there has been more than 800% increase in whole grain food introductions worldwide according to the Whole Grains Council (Anonymous 2007). According to a major industry source, in North America alone, the market for this food product category was estimated at over $5 billion in 2005 (Anonymous 2005). Clearly, the increasing popularity of whole grain foods reflects the gaining importance with consumers of products that improve diet, nutrition and health.

Due to its distinct composition of bioactive components, consumption of whole wheat has been shown consistently to be associated with reduced risk of heart disease (Anderson 2002, Slavin 2007), type 2 diabetes (Kabir et al 2002, Liu 2002), gastrointestinal cancer (Jacobs et al 1999, McIntosh 2007), and obesity (Jenkins et al 2002, Jensen et al 2004, Venn et al 2004). The health-related functionality of whole wheat is largely due to the bran fraction and bioactive constituents such as fibre and/or fibre-related constituents including phenolic compounds and phytic acid (Yu et al 2002). Many phenolic compounds have potent antioxidant activities that prevent oxidative damage of biologically important molecules in human cells, such as DNA, proteins, and lipids. This may be the basis of the connection between whole-grain or bran consumption and reduced risk of chronic disease (Jensen et al 2004, Zhou et al 2004, Liu 2007). The predominant phenolic antioxidant in wheat bran is ferulic acid which exists in free and bound forms (Onyenho et al 1992). Antioxidant activity has also been ascribed to phytic
acid or inositol hexaphosphate (Graf et al 1987, Graf and Eaton 1990, Martinez-Tome et al 2004) which is known to have potent cancer preventive properties (Midorikawa et al 2001, Somasundar et al 2005).

What differentiates wheat bran from the corresponding bran of other popular cereal grains such as oats and barley is the nature of the fibre or non-starch polysaccharide (NSP) component. Unlike β–glucans which are the main NSPs of barley and oats, wheat is distinguished by its very low content of β–glucans and abundant content of pentosans or arabinoxylans which are especially plentiful in wheat bran (from 22-28%, Wang et al 2006). Pentosans, along with the NSP β–glucan and cellulose, originate in cell walls which represent the principal dietary fibre source of all cereal grains. Arabinoxylans are NSP polymers composed of two monosaccharides with a structure comprising a xylose backbone with arabinose side chains. Arabinoxylans (AX) consist of a β-(1→4)-linked D-xylopyranose polymer backbone with frequent branching of L-arabinofuranose residues at O-3 or O-2 and O-3 (Wang et al 2006). AX polymer contains the phenolic compound ferulic acid which is esterified to arabinose residues at O-5 (Wang et al 2006). AX possesses antioxidant functionality that arises from the presence in its structure of the phenolic compound, ferulic acid. Ferulic acid is the main cell-wall bound phenolic acid in all cereal grains and, in wheat, is found almost exclusively associated with AX. Also unlike β-glucans, pentosans are known to have a distinct prebiotic function (Crittenden et al 2002), i.e. they promote the proliferation of beneficial bacteria such as Lactobacillus and/or Bifidobacterium species (Karppinen et al 2000, Yuan et al 2005, Neyrinck et al 2008, Vardakou et al 2008) which are related to good colonic health in the fermentation of dietary fibre during food digestion.
In spite of all the potential and realized health benefits of wheat bran and whole wheat products, their relative acceptance by consumers remains low compared to products manufactured from refined wheat flour (Bakke and Vickers 2007). From this, it can be surmised that many consumers dislike the different sensory properties of wheat bran and whole wheat products on account of their strong and different aroma and flavour. Whole wheat products are also challenging to manage from a processing perspective. The addition of bran to a refined bread flour formula significantly changes and negatively affects the rheological properties of the dough (Shogren et al 1981, Nelles et al 1998, Zhang et al 1999). While water absorption can be substantially enhanced by bran addition due to the added hygroscopic fibre, doughs are typically difficult to handle, are often sticky, and lack tolerance to normal processing conditions and deviations (e.g. over-mixing) in a commercial production environment. Bread formulations fortified with wheat bran characteristically produce breads with lower loaf volume (Shogren et al 1981, Lai et al 1989a), coarser crumb grain and darkened crumb color (Shogren et al 1981, Nelles et al 1998, Zhang et al 1999) and poorer bread texture (Shogren et al 1981, Salmenkallio-Marttila et al 2001). The addition of bran dilutes gluten protein which appears to be one of the main “passive” reasons why bran is detrimental to the bread making quality of whole wheat. In addition, the different physical and/or chemical nature of the bran itself may also contribute an active factor in its diminished performance.

A moderate amount of research has been reported on minimizing these negative processing attributes of wheat bran. In this regard, bran has been subjected to various treatments prior to its incorporation into dough such as hydration (Nelles et al 1998), fermentation (Salmenkallio-Marttila et al 2001), heat treatment (de Kock et al 1999) and

Improvements from these treatments were reported to be generally small or insignificant on processing properties, and outcomes not well explained. The question arises, which is the subject of this thesis research, whether these or any other bran treatments can favourably influence the solubility of wheat bran to increase the antioxidant activity of extracts and/or the extractability of fibre and other bran components.

Considerable research has been done on the antioxidant activity of whole grain cereal including wheat and bran with the bulk comprising studies of a comparative analysis nature (Beta et al 2005, Shahidi et al 2006, Liu et al 2007). Moreover, virtually all treatments of wheat or bran subjected to antioxidant activity analysis have involved in the first step, extraction using organic solvents such as petroleum ether, methanol or acetone (Yu et al 2004, Liyana-Pathirana and Shahidi 2006, Kim et al 2006), i.e. very few aqueous preparations for food-grade applications have been studied. Surprisingly as well, there appears to be very little science on enhancing the functionality of bran of wheat or other grains via pre-treatments. Even though the research has shown that wheat bran is rich in antioxidants when extracted with organic solvents, antioxidant bioavailability is not well studied (Kuijsten et al 2005, Price et al 2008, Anson et al 2009). Regardless, because all digestive secretions are aqueous in nature, an underlying rationale for this thesis research is the use of aqueous extracts of wheat bran as the starting point for analysis in order to provide a basis for subsequent research focused on bioaccessibility and bioavailability.
The objectives of this research were as follows:

1. Evaluate the effects of autoclaving conditions (high temperature, pressure and absence of O₂) compared to other thermal treatments such as air-oven and air-oven with N₂.

2. Evaluate the effects of bran particle size without and with autoclaving on dough mixing properties and extraction or solubilisation of antioxidants.

3. Evaluate in detail, the autoclaving process on antioxidant activity of water soluble extracts within the range of processing conditions available including sterilization time and temperature, and repeated treatment cycles.

4. Determine the effects of autoclaving on chemical composition of bran, water-soluble extracts and insoluble residue including composition of chemical constituents such minerals and B vitamins, protein, starch, phytic acid, β-glucans, arabinoxylans, and soluble dietary fiber and total dietary fiber.

In principle, if the water extractability and/or solubility of wheat bran can be enhanced by pre-treatment, it stands to reason that food incorporating the enhanced wheat bran will have higher levels of bioavailability of constituent bioactive compounds. The long-term goal of this research is to improve the functional properties of wheat bran in order to maximize its economic value and that of the entire grain, as well as to promote healthier food consumption by increased usage of whole wheat products.
2. LITERATURE REVIEW

2.1 Basic wheat structure and composition

Wheat grain can be portrayed in a few different ways from a botanical perspective, it can be described as a seed designed primarily to perpetuate itself. It is also the world’s most important food crop providing a rich array of leavened and non-leavened products containing important nutrients for sustaining the health and wellness of people. Consequently wheat is a predominant commercial commodity whose economic returns sustain farmers and the agri-food industry on a global basis. Whole wheat, eaten as bread and other baked products, has been a staple of the human diet for several millennia. While whole wheat’s nutritional benefits and importance has been recognized over the centuries, the use of whole wheat for food waned significantly in the early part of the twentieth century in comparison to refined wheat flour. Nevertheless, the past few decades has seen a remarkable resurgence in food products made from whole wheat and whole grains in general. This has been sparked by increasing consumer interest and awareness in healthy eating and the health protecting and promoting effects of whole wheat and other whole grains.

The nature of wheat’s value for processing and for human health derives from its composition and structure. Like all cereal grains, the wheat kernel is comprised of three major components: germ, bran and starchy endosperm (Fig. 2.1). The kernel contains a crease parallel to its long axis on its ventral side which covers about 25% of the wheat
kernel surface (Posner 2000) and affects milling properties (Evers and Millar 2002) such that the endosperm can only be freed from bran contamination by milling at relatively low flour extraction rates. Whole wheat grain can be separated more or less distinctly by conventional milling into germ, bran and endosperm, or refined flour, whose composition varies greatly (Table 2.1). The traditional economic value of wheat resides with most quantitatively predominant fraction, i.e. endosperm, which is rich in energy as it is predominantly comprised of starch. Based on careful kernel dissections of four wheats, wheat endosperm, bran (pericarp, testa and aleurone) and germ (embryo and scutellum) were found to represent about 83%, 14-15% and 2.5-3.6% by weight of the whole grain (Hinton 1959).

From the seed perspective, the germ contains the embryo, while the endosperm provides energy and nutrients for the growing seedling upon germination. Bran tissues on the other hand function as barriers to protect the seed from pests and adverse weather. The aleurone portion of the bran is also site of synthesis of $\alpha$-amylase and other enzyme which occurs upon germination. The function of these enzymes is to hydrolyse nutrients from the endosperm (Repellin et al 2007).

In descending order of content, whole wheat is composed of carbohydrates, protein, lipid, minerals (ash), and a phytochemical portion which, quantitatively, is in very low concentration (Table 2.1). Most of the carbohydrates are polysaccharides comprising, starch, arabinoxylans and cellulose. The latter two components form the bulk of the fibre fraction of whole wheat. Wheat bran is distinct from refined flour and germ in its higher levels of ash, dietary fibre and vitamin content (Table 2.1). Wheat bran has very high levels of mineral content, approximately ten fold higher than that of
endosperm. On the other hand, the germ fraction also has significant levels of mineral content compared to endosperm. The majority of vitamins are concentrated in bran part of wheat kernel. Dietary fibre of whole wheat is almost exclusively concentrated in wheat bran, and germ to a lesser extent. Endosperm contains very little fibre (Table 2.1), whereas wheat bran has approximately 17 times higher levels of dietary fibre compared to endosperm and three times more fibre compared to wheat germ. Dietary fibre is clearly a major component of bran that contributes the substantial health functionality ascribed to whole wheat.

Figure 2.1. Longitudinal and transverse sections of a wheat kernel (Dexter and Sarkar 2004).
Wheat is a globally important agricultural commodity and primary food ingredient because it is the only cereal grain capable of being processed into leavened bread, an attribute that derives from its unique gluten protein composition. Because its consumption as bread and other foods is so significant, whole wheat has the potential to deliver to consumers considerable levels of beneficial nutrients and bioactive functional food compounds. The latter components in wheat are generally qualitatively similar across cereal grains (Henry 1985), but specific proportions can be very different (Shahidi 1996, Handleman et al 1999, Peterson 2001, Philava et al 2001, Spiller 2001, Kris-Etherton et al 2002, Wood et al 2002, Martinez-Tom et al 2004). For example, as mentioned above, wheat β-glucan content is very much lower than that of oats and barley. On the other hand pentosan or arabinoxylan content is much higher in wheat (Henry 1985).

Table 2.1. Proximate composition of different layers of whole wheat grain.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Whole wheat (%)</th>
<th>Refined flour (endosperm %)</th>
<th>Wheat bran (%)</th>
<th>Wheat germ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>10.3</td>
<td>11.9</td>
<td>9.9</td>
<td>11.1</td>
</tr>
<tr>
<td>Protein</td>
<td>13.7</td>
<td>10.3</td>
<td>15.6</td>
<td>23.2</td>
</tr>
<tr>
<td>Total lipid</td>
<td>1.9</td>
<td>0.98</td>
<td>4.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Ash (minerals)</td>
<td>1.6</td>
<td>0.47</td>
<td>5.8</td>
<td>4.2</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>72.6</td>
<td>76.3</td>
<td>64.5</td>
<td>51.8</td>
</tr>
<tr>
<td>Total dietary fibre</td>
<td>12.2</td>
<td>2.7</td>
<td>47.8</td>
<td>13.2</td>
</tr>
<tr>
<td>Sugars</td>
<td>0.41</td>
<td>0.27</td>
<td>0.41</td>
<td>0</td>
</tr>
<tr>
<td>Vitamins</td>
<td>0.11</td>
<td>0.01</td>
<td>0.09</td>
<td>0.01</td>
</tr>
</tbody>
</table>


Whereas wheat endosperm contains less than 10% of the nutritive value (minerals, vitamins, dietary fibre) of whole grain, the bran fraction of the kernel contains nearly all the bioactive compounds of wheat. During last few decades, scientific and
consumer attention to wheat bran and whole wheat has increased substantially due to their importance in human health. This review will focus on the composition of wheat bran particularly from the standpoint of its phytochemical and bioactive components, especially those with antioxidant potential.

2.2 Wheat bran structure and composition

Bran represents the outermost portion of the grain which surrounds the starchy endosperm, and it can be divided into different tissue layers which are mostly comprised of cells walls. Those layers encompass the outer and inner pericarp, seed coat (testa, spermoderm, and integument), nucellar epidermis (hyaline layer, perisperm) and the nucellar projection, while the inner tissue is composed of aleurone cells (Fig. 2.1). The aleurone layer consists of living tissues generally one cell thick in wheat, surrounding the endosperm. As it is removed during milling, it constitutes the inner most layer of bran (Peyron et al 2003). Botanically, the aleurone layer of wheat is part of the endosperm, but because of its high adherence to the pericarp, aleurone is mainly found in bran fractions after milling (Evers and Bechtel 1988). Wheat as noted has a single-cell aleurone layer, whereas barley aleurone has two to four cell layers. It has been suggested that the greater number of aleurone cell layers in barley contributes to higher levels of phenolic acids, especially ferulic acid (Evers et al 1999).

Bran constitutes approximately 14-15% of total wheat by weight (Hinton, 1959, Kent et al 1975, Barron et al 2007). The thicknesses of bran’s tissue layers vary (Barron et al 2007). The aleurone is the thickest layer (up to 65 µm). The outer pericarp is of intermediate thickness (15–30 µm), and the seed coat is the thinnest (5–8 µm). Variation
in bran thickness in an individual wheat grain is believed to be due to the irregularities of thickness of aleurone cells affecting the separation of bran from endosperm during milling (Crewe and Jones 1951). Thus, flour yield during milling depends in part on bran thickness, size of grain (bran and endosperm content) and crease characteristics (Corke 2004). An interesting question is whether reported differences among wheats in antioxidant activity (Adom et al 2003, Beta et al 2005, Moore et al 2006) are due to variation in thickness of the aleurone layer, and therefore concentration of constituent phytochemicals such as ferulic acid. There appears to be no science to answer this question at the present time.

It is important to point out that commercial wheat bran is not same as botanical bran, i.e. as it exists in the original grain. Wheat bran can be derived from various stages of the milling process and, depending on the set-up of the mill in terms of flour extraction, can vary in purity, i.e. endosperm contamination, and hence can vary in its content of fibre, protein and other constituents, due to milling effects alone, and also due to the type of wheat used, and environmental conditions during wheat kernel development (Posner 2000). Wheat bran can be derived from conventional dry roller milling or a combination of conventional milling and abrasion processing similar to that used in rice polishing (Tkac 1992, Fulcher and Rooney 2002, Pandiella et al 2004). In roller-milling, wheat bran is typically derived as the coarse by-product passing over, or “over-tailing”, the top sieve (~ 1.3 mm aperture) of the final break roll (Ziegler and Greer 1971) and is considered to be free of germ. Ziegler and Greer (1971) reported bran yields of 5%, 7% and 10% for straight-run flour extractions of 85%, 80% and 70%,
respectively. In the present study, wheat bran yield was 11% from pilot milling to about 75% extraction.

Wheat bran and its aleurone component in particular have important biological functions, both physical and chemical, to protect the grain from molds, bacteria, insects and severe weather conditions that could damage the seed (Fulcher and Rooney- Duke 2002). For example, wheat bran phenolic acids present in cell walls are thought to have an important role to cross-link polysaccharides with other cell wall components including lignin, and also in the cross-linking of polysaccharide chains (Parker et al 2005), thereby increasing the integrity of cell walls and may therefore provide structural resistance to invading fungi and other microorganisms (McKeehen et al 1999). The testa (seed coat) that separates the pericarp from bran’s more internal tissues and endosperm appears to be chemically inert and waterproof (Briggs 1974). Furthermore the seed coat reduces the inward penetration of microorganisms which are almost always are present in the pericarp of wheat bran (Briggs 1974).

From a functional food perspective, the aleurone is the most significant tissue in the wheat kernel as it contains high levels of protein, lipid, vitamins, minerals and phytochemicals. The aleurone layer is rich in phenolic compounds such as ferulic acid and $\rho$-coumaric acid (Smith et al 1983, Greffuille et al 2005) and therefore contributes the highest antioxidant capacity of any wheat tissue fraction (Anson et al 2008). Aleurone cells are also rich in niacin, lysine and soluble B vitamins (B1, B2, B3, B6, B9), minerals (P, K, Mg, Mn, Fe) (Laubin et al 2008), plus betain and choline (Graham et al 2009). In addition, wheat aleurone has a very high content of phytic acid in the form of phytate (Hemery et al 2009).
2.2.1 Wheat bran fibre composition

Dietary fibre is defined as the “edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human intestine with complete or partial fermentation in the large intestine” (AACC International 2005). Dietary fibre includes oligosaccharides, cellulose, lignin, pectin and other polysaccharides such as β-glucan and arabinoxylans. The most important fibre components in wheat are the non-starch polysaccharides (arabinoxylans, β-glucan and cellulose) and the non-polysaccharide, lignin (Gebruers et al 2008). Non-starch polysaccharides (NSP) can have major effects on the functionality of cereal grains for food even though they account for only about 3-8% of whole grain (Saulnier et al 2007). In wheat, arabinoxylans and mixed-linkage (1-3)(1-4)-β-D glucans are the main components of the endosperm cell walls. However the distribution of these compounds can vary between wheat and other cereal grains. In oats and barley, endosperm cell walls contain approximately 75% mixed-linkage (1-3)(1-4)-β-D glucans and only 15% of arabinoxylans (Miller et al 1995), whereas wheat endosperm cell walls contain approximately 75% arabinoxylans and 15% mixed-linkage (1-3)(1-4)-β-D glucans (Mares and Stone 1973, Basic and Stone 1980).

What differentiates wheat bran from the corresponding bran of other cereal grains such as oats and barley is the nature of the fibre or NSP component. Unlike β–glucans which are the main NSPs of barley and oats, wheat is distinguished by its content of pentosans or arabinoxylan which are especially abundant in wheat bran; 22-28%, (Wang et al 2006). Accordingly, Arabinoxylans are quantitatively the most important wheat bran polysaccharide and represents up to 60-70% of the total fibre fraction.
2.2.1.1 Arabinoxylans

Arabinoxylans originate in the cell walls of endosperm and bran tissues. Pentosans, a term still used in the current literature synonymous with arabinoxylans (AX), represent NSPs composed of five-carbon or pentose monosaccharides. More precisely, AXs are the predominant constituent of pentosans, and consist of β-(1-4)-linked D-xylopyranose polymer backbone with frequent branching of L-arabinofuranose residues at O-3 or O-2 and O-3 (Gruppen et al 1992, Wang et al 2006). Significantly from the standpoint of AX structure and bioactivity, AX polymer also contains the phenolic compound ferulic acid which is esterified to arabinose residues at O-5 (Wang et al 2006). Ferulic acid is the main cell-wall bound phenolic acid in all cereal grains and in wheat it is found almost exclusively associated with AX or pentosans (Izydorczyk et al 1995, Cui et al 2009). Dehydroferulic acid dimers (diferulates) have an important structural role to cross link and therefore stabilize AX polysaccharides (Hartley et al 1990), and hence cells walls. Consequently, diferulates may be partly responsible for the insoluble nature attributed to the dietary fibre of wheat bran (Bunzel et al 2001). As well, ferulic acid is the key component involved in oxidative gelation of pentosans (Fausch et al 1963), a cross-linking reaction believed to be unique to water-extractable pentosans (WEP) (Hoseney and Faubion 1981). Importantly, the antioxidant potency of wheat is predominantly determined by the content of ferulic acid in bran and the aleurone fraction in particular (Table 2.2) (Anson et al 2008).

WEP and water-unextractable pentosans (WUP) comprise ~25% and 75%, respectively, of total pentosans present in wheat (Meuser and Suckow 1986). Water-soluble pentosans are constituted mainly of AX but also contain some arabinogalactans. The remaining pentosans are water-insoluble and are mainly composed of AX (Faurot et
The behaviour of pentosans in aqueous solutions is believed to relate to the shape and size of the polymers, degree of substitution and contiguity of substitution of arabinose molecules (Gruppen et al 1993). In general, a higher ratio of arabinose (A) to xylose (X) is associated with higher solubility of AX in water. Average total AX and WE-AX contents in whole wheat are 6.7 and 0.7% (14% moisture basis), respectively (Wang et al 2006).

The layers of wheat bran (aleurone and pericarp) and germ are much richer in AX than the starchy endosperm, with the highest levels (~40%) occurring in the outer pericarp (Barron et al 2007). Whereas relatively low A/X values have been reported for aleurone (~0.39) and the intermediate layers A/X (~0.36) compared to starchy endosperm A/X (~0.85, Barron et al 2007), outer pericarp AX is more heavily substituted and is high in A/X ~1.14 (Table 2.2, Izydorczyk et al 1995, Saulnier et al 2007, Gebruers et al 2008). AX in the peripheral layers, especially in the pericarp, also contains uronic acid residues (Saulnier et al 2007, Gebruers et al 2008). Covalent ester bonding between the carbonyl group of uronic acid and hydroxyl group of AX, as well as formation of diferulate bridges between adjacent AX chains which make them insoluble in water (Izydorczyk et al 1995), all likely contribute to the general insolubility of wheat bran and the high degree of indigestibility attributed to this fibre compound.

Apart from its nutritional relevance, AX is also important from a technological point of view as it strongly affects wheat functionality during processing, especially breadmaking via its disproportionally high water binding capacity (Gebruers et al 2008).

Even though ferulic acid is an important structural element of AX, the amount linked to AX is very low and represents 0.2-0.4% of WE-AX and 0.6-0.9% WU-AX in
wheat (Cui et al 2009). This corresponds to about 2 to 4 and 10 to 16 ferulic acid residues per 1000 xylose residues in WE-AX and WU-AX, respectively. Dehydroferulic acid dimers were also detected in very low amounts.

Table 2.1. Composition of wheat bran tissues.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>AX (g/100g)</th>
<th>Glucose (g/100g)</th>
<th>Uronic acid (g/100g)</th>
<th>A/X (mg/g)</th>
<th>Ferulic acid (mg/g)</th>
<th>Ferulic acid dehydrodimers (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer pericarp</td>
<td>44.1</td>
<td>29.3</td>
<td>nd</td>
<td>1.14</td>
<td>3.7</td>
<td>4.1</td>
</tr>
<tr>
<td>Inner pericarp + testa+ NE³</td>
<td>38</td>
<td>17.6</td>
<td>nd</td>
<td>0.36</td>
<td>5</td>
<td>0.91</td>
</tr>
<tr>
<td>Inner pericarp</td>
<td>49</td>
<td>15.8</td>
<td>9.5</td>
<td>1.06</td>
<td>~1</td>
<td>~0.5</td>
</tr>
<tr>
<td>Testa + NE</td>
<td>55</td>
<td>3.8</td>
<td>3.2</td>
<td>0.13</td>
<td>~5</td>
<td>~0.3</td>
</tr>
<tr>
<td>Aleurone</td>
<td>20.8</td>
<td>15</td>
<td>nd</td>
<td>0.39</td>
<td>6.7</td>
<td>0.95</td>
</tr>
</tbody>
</table>

¹Data source: Saulnier et al (2007)  
²nd, not determined  
³NE, nuclear epidermis

2.2.1.2. β-glucan

β-glucans (beta-glucans) are β-linked polysaccharides of D-glucose monomers. β-glucans are a diverse group of molecules which can vary with respect to molecular mass, solubility, viscosity, and three-dimensional configuration. Cereal β-glucan occurs in the sub-aleurone and endosperm cell walls of the seeds of cereals, including oats, barley, rye and wheat. In oats, β-glucans are concentrated in the sub-aleurone layer, while in barley and rye, β-glucans are located mainly in the endosperm. The literature regarding the precise distribution of β-glucans in the wheat kernel is incomplete (Cui and Wang 2009). It is believed that β-glucans are concentrated in the walls of sub-aleurone cells as evidenced by the enrichment of β-glucans in the bran fractions using debranning.
processes (Dexter and Wood 1996, Cui et al 2000). The β-glucan levels of wheat are low (0.5-1%, Cui and Wang 2009), and represents <1% of the total dietary fibre content of whole wheat (Cui et al 1999).

The molecular weight of β-glucan in wheat is reported to be lower than that of barley and oats. Hence, the extractability and functionality of β-glucan in wheat is believed to be higher than that in oats and barley (Cui and Wood 2000), however because of its low concentration in wheat, β-glucans likely contributes little by way of health attributes, compared to oats and barley. In these latter grains, β-glucan is considered to be an important soluble fibre that is associated with lowering serum cholesterol levels and attenuating glycemic response (Wood et al 1994). These effects originate in the upper gastrointestinal tract and probably are related to the viscosity of β-glucan (Wood et al 2002).

2.2.1.3. Cellulose

Cellulose is a very abundant cell wall polysaccharide in the outer pericarp and intermediate layers of wheat bran. Cellulose consists of glucose units linked together by β-1-4 glycosidic bonds to form a linear polysaccharide (Lineback et al 1988). Cellulose comprises about 21% of wheat bran on a 14% moisture basis; however estimates vary widely (Atwell 2002). Cellulose is considered to be very resistant to intestinal microbial fermentation when compared with the non-cellulosic fibre polysaccharides such as AX and β-glucans. Literature values for the digestibility of cellulose vary considerably depending on the source of cellulose or the type of food containing cellulose and its concentration. A study using pigs consuming whole wheat bread as feed reported ileal
digestibilities of cellulose, AX, β-glucan and starch of 19%, 28%, 73% and 98%, respectively (Gall et al 2009).

2.2.1.4 Lignin

Lignin is a complex polyphenolic polymer residing in the pericarp cell walls and the seed coat cell walls of wheat bran (Ferguson 1999). Lignin is a non polysaccharide cell wall substance that is mainly derived from the three monolignols: ρ coumaryl, coniferyl and synapyl alcohols (Gebruers et al 2008). Lignins are polar polymers with an average of one to two hydroxyl groups per monomer and have very poor solubility in non-polar solvents (Pouteau et al 2003). The structure of lignin depends on its botanical origin, environmental conditions of crop growth and also conditions of extraction (Pouteau et al 2003). Whole wheat has a lignin concentration of ~2% of dry material (Ferguson 1999). The distribution of lignin in wheat has been reported to range from 2.8-3.7%, 1.2-3.8%, 2.3-7.7% and 4.1-8.7% in bran, outer pericarp, inner pericarp and seed coat respectively (Schwarz et al 1988). In contrast, aleurone cell walls of wheat bran contain no lignin (Ferguson and Harris 1999). Ferulic acid, together with dehydroferulic acid, is a component of lignocelluloses, serving to crosslink the lignin and polysaccharide, thereby conferring rigidity to the cell walls (Jiyama et al 1994). Ferulic acid is most often linked by ester bonds to arabinose residues of AX, but in lignin it often polymerizes through ether bonds to AX (Klepacka and Fornal 2006).

2.2.1.5 Fructans

Fructans are a category of functional food carbohydrates that encompass naturally occurring plant oligosaccharides and polysaccharides containing fructose. Fructans are different in nature and classified according to linkage and origin. Gramineae type
fructans that can be found in wheat contains β-(1-2) and β-(6-2) linkage (Haska et al. 2008). Fructooligosaccharide (FOS) is a subgroup of inulin, consisting of linear chains of fructosyl monomers linked by β-(1-2) bonds with a degree of polymerization ≤10. Whole wheat grain fructan content has been reported (Haska et al. 2008) as 0.9-1.8 g/100 g, whereas wheat bran and middlings had higher levels of 2 g/100 g and 2.3 g/100 g, respectively, compared to 1.6 g/100 g in wheat flour.

2.2.2. Phytochemical composition of wheat bran

In addition to fibre components discussed above, whole wheat is rich in bioactive compounds. Wheat bran is even richer. Table 2.3 presents a detailed comparison of the composition of non-nutrients and functional components in whole wheat and bran. Wheat germ and bran in particular contain the majority of bioactives in the grain. The bioactive constituents of bran encompass the total fibre fraction which includes AX as wheat’s principle NSP (Martinez-Tome et al. 2004). Wheat bran also contains significant quantities of oligosaccharides such as fructans and inulin. In addition to minerals (not shown in Table 2.3) wheat bran also enhances whole wheat bioactive composition in terms of phenolic compounds, alkylresorcinols, sterols, carotenoids, betaine and choline, phytic acid, flavonoids and B vitamins all which have been linked to positive health efficacies (Smith et al. 1983, Shahidi 1996, Handleman et al. 1999, Zielinski and Kozlowska 2000, Peterson 2001, Pihlava et al. 2001, Kris-Etherton et al. 2002, Graham et al. 2009).
Table 2.2. Composition of phytochemicals in whole wheat and bran.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Whole grain</th>
<th>Bran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-glucan (%)</td>
<td>0.4-1.4&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arabinoxylan (%)</td>
<td>5.68-8&lt;sup&gt;3&lt;/sup&gt;</td>
<td>8.9-28.0&lt;sup&gt;4,5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inulin /fructan (g/100g)</td>
<td>2.3&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3.4-4.4&lt;sup&gt;6,7&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>2&lt;sup&gt;7&lt;/sup&gt;</td>
<td>32.2&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td>1.40-3.25&lt;sup&gt;4,9&lt;/sup&gt;</td>
<td>5.2&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lignan (µg/g)</td>
<td>35.5&lt;sup&gt;10&lt;/sup&gt;-711&lt;sup&gt;11&lt;/sup&gt;</td>
<td>110&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phenolic acid (µg/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferulic</td>
<td>640&lt;sup&gt;12&lt;/sup&gt;-1270&lt;sup&gt;13&lt;/sup&gt;</td>
<td>1942&lt;sup&gt;13&lt;/sup&gt;-5410&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.6&lt;sup&gt;13&lt;/sup&gt;-35&lt;sup&gt;13&lt;/sup&gt;</td>
<td>100&lt;sup&gt;13&lt;/sup&gt;-164&lt;sup&gt;13&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sinapic</td>
<td>1.3&lt;sup&gt;13&lt;/sup&gt;-63&lt;sup&gt;13&lt;/sup&gt;</td>
<td>170-300&lt;sup&gt;13&lt;/sup&gt;</td>
</tr>
<tr>
<td>〈p- coumaric</td>
<td>0.2&lt;sup&gt;13&lt;/sup&gt;-37.2&lt;sup&gt;13&lt;/sup&gt;</td>
<td>100&lt;sup&gt;13&lt;/sup&gt;-457&lt;sup&gt;13&lt;/sup&gt;</td>
</tr>
<tr>
<td>Syringic</td>
<td>-</td>
<td>29.27-85.01&lt;sup&gt;14&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alkylresorcinols (µg/g)</td>
<td>317-1429&lt;sup&gt;8,29&lt;/sup&gt;</td>
<td>2211&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sterols (mg/100g)</td>
<td>59.2&lt;sup&gt;15&lt;/sup&gt;-74.4&lt;sup&gt;10&lt;/sup&gt;</td>
<td>150.3&lt;sup&gt;15&lt;/sup&gt;-195.1&lt;sup&gt;10&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carotenoids (µg/g)</td>
<td>1.48-2.71 (DW)&lt;sup&gt;16&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Zeaxanthine</td>
<td>2.7&lt;sup&gt;17&lt;/sup&gt;</td>
<td>23&lt;sup&gt;17&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lutein</td>
<td>26.41-143.46&lt;sup&gt;18&lt;/sup&gt;</td>
<td>50-180&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
<tr>
<td>β cryptoxanthin</td>
<td>1.0-13.5&lt;sup&gt;18&lt;/sup&gt;</td>
<td>18-64&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
<tr>
<td>β carotene</td>
<td>5&lt;sup&gt;20&lt;/sup&gt;</td>
<td>6&lt;sup&gt;20&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tocopherol (mg/100g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tocopherol</td>
<td>2.3&lt;sup&gt;21&lt;/sup&gt;</td>
<td>0.8-1.2&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tocotrienol</td>
<td>3.7&lt;sup&gt;21&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Betaine (mg/100g)</td>
<td>291.2&lt;sup&gt;22&lt;/sup&gt;</td>
<td>867&lt;sup&gt;23&lt;/sup&gt;, 1505.60&lt;sup&gt;11&lt;/sup&gt;</td>
</tr>
<tr>
<td>Choline (mg/100g)</td>
<td>27.3&lt;sup&gt;23&lt;/sup&gt;</td>
<td>50.89&lt;sup&gt;11&lt;/sup&gt;, 102&lt;sup&gt;23&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phytic acid (mg/100g)</td>
<td>906&lt;sup&gt;40&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>210</td>
<td>149.1-405.7 ug/g&lt;sup&gt;25&lt;/sup&gt;, 570 ug rutin equivalent/g&lt;sup&gt;24&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flavonoids (mg/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavanols</td>
<td>0.5-1.3&lt;sup&gt;26&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Anthocyanins (µg/g)</td>
<td>0.55-0.83&lt;sup&gt;27&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>B Vitamins (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1 Thiamin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2 Riboflavin</td>
<td>0.502&lt;sup&gt;30&lt;/sup&gt;</td>
<td>0.523&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>B3 Niacin</td>
<td>0.165&lt;sup&gt;30&lt;/sup&gt;</td>
<td>0.577&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>B5 Pantothenic acid</td>
<td>4.957&lt;sup&gt;30&lt;/sup&gt;</td>
<td>13.578&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>B6 Pyrodoxine</td>
<td>0.603&lt;sup&gt;30&lt;/sup&gt;</td>
<td>2.181&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>B9 Folate (µg)</td>
<td>0.407&lt;sup&gt;30&lt;/sup&gt;</td>
<td>1.303&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>44&lt;sup&gt;30&lt;/sup&gt;</td>
<td>79&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td>Data sources for Table 2.3:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
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</tr>
</tbody>
</table>
2.2.2.1. Wheat bran phenolic composition

Whole wheat contains various classes of phenolic compounds including phenolic acids, flavonoids, stilbenes, coumarins and tannins (Shahidi and Nazck 1995, Lloyd et al 2000). Relative content in wheat of phenolic acids and other phenolic compounds depends on the tissue or fraction type. For example, in whole wheat, phenolic acids comprise ~42% of total phenolic compounds whereas in wheat bran phenolic acids make up more than 60% of total phenolics (Zhou et al 2004, Vitaglione et al 2008). Phenolics are compounds with one or more aromatic rings containing one or more hydroxyl groups (Fig. 2.2). Phenolic compounds are the product of secondary metabolism. As discussed above, they have important structural and defence roles in grains against microorganisms, insects, parasites and adverse environmental conditions. These compounds exist as glycoside complexes linked to organic acids, amines, lipids and carbohydrates and other phenols (Liu et al 2007). Phenolic compounds exist as soluble free, soluble-conjugated (eg. esterified) and insoluble bound forms in wheat bran (Adom et al 2005). Phenolic content variation in whole wheat and bran depends on genotype and environmental conditions (Mopfu et al 2006).

![Figure 2.2. Structure of common phenolic compounds (Liu et al 2007).](image-url)

2.2.2.1 Phenolic acids

Phenolics acids can be subdivided into two major groups; hydroxybenzoic acids and hydroxycinnamic acids (Fig. 2.3). Hydroxybenzoic acids include p-hydroxybenzoic, protocatechuic, vanillic acid, synergetic and gallic acid. They are typically components of complex compounds like lignins and tannins. They can also be found as derivatives of sugars and organic acids in plant foods. Hydroxycinnamic acid includes p-coumaric, ferulic, caffeic and syringic acids which form links to cell wall structural components such as pentosans, cellulose and lignins through ester bonds (Liu 2007).

![Diagram of phenolic acids](image)

Figure 2.3. Common phenolic acids, benzoic type (A) and cinnamic acid type (B). Different phenolic acids vary depending on substitutions at R1, R2 and R3 (Adapted from Liu et al 2007).

Sosulski et al (1982) first reported the content of free, esterified and insoluble bound phenolic acids of wheat flour (debranned by roller milling). The analysis was based on quantification of individual phenolic acids by GLC-MS. Wheat flour was also analysed. Total phenolic acid content in fresh and stored wheat was 2.3, 9.1 and 60 ppm in the free, esterified and insoluble bound fractions. These values for free, esterified and bound phenolics correspond to 3.2%, 12.8% and 84%, respectively. Accordingly, free phenolic
acids constituted a very small proportion of total wheat phenolics which were predominantly in bound form. Interestingly, aged wheat flour had about 35-40% lower content of phenolic acids which was attributed to destructive oxidation reactions during storage.

Similar results were reported by Hatcher and Kruger (1997) who determined free, esterified and bound phenolic acids in whole wheat and flour streams of different degrees of refinement (i.e. bran contamination), and in whole wheat of five different wheat classes. Reversed-phase HPLC was used for phenolic acid quantification. For whole wheat, the percentage composition of free, esterified and bound phenolic acids in total phenolic acids was approximately 3%, 17% and 80%, respectively, which was very similar to proportions reported by Sosulski et al (1982). In contrast, a more recent study (Liyana-Pathirana and Shahidi 2006) reported free, esterified and bound phenolic content in two wheat genotypes of approximately 11, 30 and 58%, respectively, i.e. very different proportions compared to previous reports. The different results can be explained in part by the different extraction procedures employed. However, Liyana-Pathirana and Shahidi (2006) used a chemical assay method i.e. Folin-Ciocalteu, to determine phenolic content of extracts which appears to over-estimate free and esterified phenolic content compared to HPLC-based direct analytical approaches used in previous research.

Hatcher and Kruger (1997) showed that as wheat milling fractions became progressively enriched with wheat bran, the content of phenolic acids increased substantially; there was ~ 10 fold difference in total phenolic acid content between 1st patent flour which was essentially free of any bran (20-40 ppm depending on genotype and flour extraction), and 2nd clear flour (over 500 ppm), which is a very high bran containing millstream. Hatcher and Kruger (1997) reported total content of phenolic acids in whole wheat for a range of
genotypes of over 400 ppm which was much higher than the 75 ppm reported previously for debranned wheat (Sosulski et al 1982).

It is clear from the literature that results of quantifying total phenolic and sub-fraction (e.g. phenolic acids) content of wheat depends on the method of extraction, measurement and that inter- or even intra-lab variation can be high. For example as noted above, Liyana-Pathirana and Shahidi (2006) used a Folin-Ciocalteu reagent assay to analyze whole grain hard and soft wheats and reported average free, esterified and bound phenolic contents (calibrated to ferulic acid equivalents, FAE) of 415, 1075 and 2146 ppm, respectively, and total phenolic content of 3637 ppm; values for whole wheat were almost 10 times higher than those reported by Hatcher and Kruger (1997). Also compared to whole wheat, wheat bran had almost four times higher total phenolic content of over 14,000 ppm (FAE) or 1.4% of grain weight which underscores the significance of wheat bran as a repository of this important class of bioactive compounds. In a later paper (Liyana-Pathirana and Shahidi 2007), the same total phenolic content (TPC) assay was applied to dried crude phenolic extracts (80% ethanol) and more moderate levels of TPC were obtained for whole grain and bran of hard red spring wheat of 1291 and 3437 ppm (FAE equivalents), respectively. Presumably, a significant proportion of insoluble bound phenolics were not extracted with 80% ethanol, which would account for the lower values reported in Liyana-Pathirana and Shahidi (2006).

In perhaps the most comprehensive evaluation of phenolic content in wheat material to date, Verma et al (2008) analyzed free and bound phenolic content of over 30 wheat genotypes; TPC contents of wheat bran ranged from approximately 4600-6700 ppm (gallic acid equivalents) or less than 50% of the TPC contents in wheat bran reported by Liyana-
Pathirana and Shahidi (2006). In the latter study, 4 M NaOH was used to solubilize bound phenolics which represents a more intense ester-bond hydrolysis than the 2 M treatment used by Verma et al (2008), and consequently could account for the higher values obtained by Liyana-Pathirana and Shahidi (2006).

2.2.2.1.2. Ferulic acid

By far, the predominant phenolic acid in wheat is (trans) ferulic acid which comprised 89% of total phenolic acids in whole wheat grain (Sosulski et al 1982). In that study, syringic and vanillic acid made up the remainder of the phenolic acids which were present in significant quantities only in free and esterified fractions. In the insoluble bound fraction of total phenolics, ferulic acid is essentially the only detectable phenolic acid (Sosulski et al 1982, Hatcher and Kruger 1997).

Like all phenolic acids in wheat ferulic acid exists as free, esterified and bound forms (Hatcher and Kruger 1997, Shahidi et al 2006). Insoluble bound ferulic acid is present in significantly greater amounts compared to free and soluble-conjugated ferulic acid in wheat (Labat et al 2000, Adom et al 2005). Ferulic acid is predominant in wheat aleurone, pericarp and embryo cells, while trace amount can be found in starchy endosperm (Sosulski et al 1982, Liu et al 2007).

Saulnier et al (2007) and Anson et al (2008) studied the distribution of ferulic acid in different hand-dissected fractions of whole wheat, especially different layers of bran (Table 2.2). Aleurone cells were clearly associated with the highest concentration of ferulic acid (Saulnier et al 2007, Anson et al 2008, Parker et al 2005) as well as antioxidant activity
(Anson et al 2008). Parker et al (2005) also showed that the seed coat or testa layer of wheat bran contained significant quantities of ferulic acid, much higher than those in the pericarp.

Total ferulic acid concentration in what bran has been reported to be four times higher than that of whole wheat grain (Yu and Cheng 2007). Ferulic acid content also significantly differs between cultivars and different growing locations of wheat varieties (Abdel-Aal et al 2001, Adom et al 2003).

Over 95% of the total phenolic acids of the bran layers are represented by ferulic acid (4-hydroxy-3 methoxy cinnamic acid) and of oxidatively coupled products, the diferulic acids (also named dehydrodimers), that are ester-linked to the cell wall polymers (Andreasen et al 2001, Antoine et al 2003). The dehydrodimers of ferulic acids are important structural components in the plant cell wall and serve to enhance its rigidity and strength (Mathew and Abraham 2004). Cross-linked AX exists due to oxidative coupling of ferulate esters in plant cell walls via peroxidase action, creating diferulic acids (Garcia et al 2002). Dimerisation of ferulates is possible by photochemical coupling reactions or radical coupling reactions. Ferulates and dehydrodiferulates have a significant role in cross-linking polysaccharides to lignin (Bunzel et al 2001). Diferulic acid levels in wheat bran have been reported to be as high as 0.01% (Andreason et al 2001). Ferulic acid has strong antioxidant potential due to its resonance-stabilized phenoxy radical structure (Adom et al 2002). The mechanism behind the action of ferulic acid lies mainly due to its free radical scavenging capability. In that respect, ferulic acid’s antioxidant capacity appears to be at an intermediate level compared to other plant phenolic acids present in wheat, e.g. syringic and vanillic acids, ferulic acid has free radical scavenging capacities at least 25% higher as assessed by a variety of methods (Yu and Cheng 2008).
2.2.2.1.3. Flavonoids

Flavonoids represent a large group of phenolic compounds that occur widely in plants (Ferguson et al 1999). Flavonoids are composed of seven major groups, viz. aurones, chalcones, flavones, flavonols, flavandiols, anthocyanins and proanthocyanins, which are synthesized through the flavonoid biosynthesis pathway (Winkel-Shirley, 2001). Not all of these flavonoid types are found in wheat grain. In wheat grain, flavonoids are mainly in glycosidic and acylglycosidic form: two apigenin-C-diglycosides and their Wessely-Moser isomers, together with their sinapic acid esters (Abdel-Aal and Hucl 2003, Asenstorfer et al 2006).

Flavonoids of wheat grain have been found predominantly in the germ (2746.3 naringin equivalents mg/100 g, Pieterzak and Collins 1996, Asenstorfer et al 2006) and smaller amounts (14.9-40.5 mg/100 g) have been reported in wheat bran (Feng and McDonald 1989). However, reported flavonoids values for wheat germ are for pure germ and in wheat kernel, germ represent ~3% of kernel weight whereas bran represents 14-15% of kernel weight.
An important type of flavone compound in wheat grain is apigenin or apigenin-C-diglycoside. These compounds are synthesized in the germ, but can be detected in refined flour after milling (Asenstorfer et al 2006). They are potentially useful antioxidants and important components in the development of the yellow colour of Asian alkaline noodles (Asenstorfer et al, 2006).

Red pigment in the seed coat tissue is a derivative of catechins, probably, phlobaphene or proanthocyanidin (Miyamoto and Everson 1958, Himi et al 2005). White grained wheat has lesser amounts of proanthocyanidins in their seed coats than red wheat (Matus-Cadiz et al 2008). Chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H) and dihydroflavanol reductase (DFR) are enzymes that catalyse the flavonoid synthesis pathway and have a reduced role in white wheat as compared to red wheat (Winkel-Shirley 2001, Himi and Noda 2005, Matus-Cadiz et al 2008). Research has shown a positive relationship between catechin tannin content and seed coat color in immature kernels, with red, light red and white varieties showing the highest to lowest levels, respectively. The precursors of brown pigment in red wheat are believed to be either phlobaphene or proanthocyanidin (Miyamoto and Everson 1958). Feng and McDonald (1989) reported that red-branned wheats contained lower levels of total flavonoids in roller milled bran compared to white common wheat and durum wheat that contains no pigment in the seed coat. Accordingly, total flavonoid does not appear to be correlated with wheat bran color. More recent work (Himi et al 2005) found that the expression of early genes of the flavonoid biosynthesis pathway was almost completely suppressed in the developing grains of white-grained wheat, compared with that in red-grained wheat. The authors concluded that wheat grain pigment was primarily proanthocyanidin rather than phlobaphene. Regardless of
the specific compound, evidently the bran tissues of white and red wheat contain differences in specific flavonoid composition.

Anthocyanins are another major flavonoid component that is predominantly found in blue and purple-coloured wheat bran. Tsao (2008) reported total concentration of anthocyanin in the bran of blue and purple wheat of 235 and 425 µg/g, respectively. By comparison, red-branned wheat contained relatively little anthocyanin content (10 µg/g). Abdel-Aal and Hucl (1999) reported anthocyanin content in pigmented wheat experimental lines ranging from 50-500 µg/g, with mean content of approximately 180 µg/g.

There appears to be a relationship between wheat bran colour, total phenolic content and antioxidant activity (Verma et al 2008) when mean values of many genotypes of a given class are compared, but not within genotypes of a given wheat class.

2.2.2.1.4. Lignans

Lignans are a group of polyphenolic compounds in plants that form oxidative coupling of two \( \rho \)-propylphenol molecules. It is believed that the best food source of plant lignan is flax (Tsao 2008), however wheat bran has also been reported to contain similar levels (Smeds et al 2007). Lignan content in wheat and other cereals varies probably due to grain size, genotype and growing location (Smeds et al 2007). In wheat, bran contains significantly higher levels of lignan concentration (~7500 µg/100 g bran, Smeds et al 2007) compare to whole wheat grain (340-2270 µg/100 g, Smeds et al 2009).

Syringaresinol is probably the predominant lignan in whole wheat grain. Its contribution to the total lignan content was approximately 80% (Smeds et al 2007). However, Mazur et al (1998) reported secoisolariciresinol diglycoside (SDG) as the dominant lignan in
wheat bran. In addition, six different lignan aglycones have been identified in wheat bran which in total accounted for 2774 µg/100 g of bran (Tsao 2008). A few studies (Drankhan et al 2003, Qu 2005) have suggested that lignans may contribute, at least in part, often noted cancer preventive properties of wheat bran.

2.2.2.1.5. Alkylresorcinols

Alkylresorcinols (AR) are a group of phenolic lipids which are present exclusively in the outer cuticle of the testa or seed coat tissue in wheat (Aman et al 2007). AR consists of a phenolic ring with two hydroxyl groups in the meta position, and an odd numbered alkyl chain at position 5. The alkyl chain in cereal AR varies from 15 to 25 carbons and is mostly saturated. It is reported that AR are exclusively found in the bran fraction of wheat, i.e. refined flour contains no or negligible amounts of ARs (Ross et al 2003, Landberg et al 2008, Athukorala et al 2010). Wheat bran has been reported to contain AR levels of 32-101 mg/100 g (Athukorala et al 2010, Ross et al 2003). Hand-dissected wheat fractions showed that over 99% of total AR are concentrated in the intermediate layer of the caryopsis, which includes the hyaline layer, testa and inner pericarp (Landberg et al 2008). Several studies have reported that AR exhibits an ability to protect cellular lipid components from oxidative processes (Struski and Kozubek 1992, Nienartowicz and Kozubek 1993, Kozubek and Nienartowicz 1995, Winata and Lorenz 1996, Hadyszowski and Kozubek 1998).

2.2.2.2. Wheat bran non-phenolic composition

2.2.2.2.1. Carotinoids

Carotinoids are the most widely spread plant pigments in nature and contribute yellow, red and orange colors. Carotinoids can be divided in two general classes: carotene
and xanthophyls (Panfili et al 2004). Carotenoids (α and β carotene, lycopene) are a class of hydrocarbons comprising group of eight isoprene units. Xanthophylls are oxygenated derivatives of carotenes (β-cryptoxanthin, lutein, zeaxanthin). In red winter wheat, lutein is the most predominant carotinoid followed by zeaxanthin and β-carotene (Tsao 2008). It has been reported that total carotene concentration in winter wheat is typically 2-5 µg/g dry material (Zhou et al 2004), whereas Canadian durum and Australia general purpose wheat bran reported to have carotinoid contents of 0.68 µmol/100 g bran (Zhou et al 2004; Tsao 2008). Moore et al (2005) reported lutein content of 0.82-1.14 µg/g in soft red winter wheat genotypes. In another study (Adom et al 2003) wheat bran had higher levels of lutein content (1.8 µg/g) compared to whole wheat. Among different wheat grain fractions, germ had elevated levels of lutein content (5.42-5.58 mg/Kg) compared to whole wheat and wheat bran fractions (Panfili et al 2004), indicating that the richest source of lutein in wheat grain is the germ fraction. Other carotenoids (carotene and zeaxanthin) had significantly lower levels (0.05-0.25 mg/Kg) in whole wheat (Panfili et al 2004).

Carotenoids have a 40 carbon skeleton of isoprene units and may be cyclised. Due to their characteristic long series of conjugated double bonds in the central part of molecule, this provides a favourable chemical reactivity as well as light-absorbing property as an antioxidant with potentially promising effects on human health. Carotenoids are considered to be free radical scavengers (Yu et al 2004, Adom et al 2003) and have powerful activities against singlet oxygen generated through lipid oxidation and peroxidation (Liu 2007).
2.2.2.4. Tocopherols

Vitamin E is an important antioxidant in cereal grain and is composed of eight isomers in nature: four tocopherols (α, β, δ and γ tocopherols) and four tocotrienols (α, β, δ and γ tocotrienols, Engelsen and Hansen 2009). Among those, β-tocotrienol is the dominant vitamer found in wheat (33-43 µg/g, Hidalgo et al 2006). It has been reported that, the total vitamin E content of wheat germ varies from 152-252 µg/g, while wheat bran vitamin E content varies from 14-75 µg/g (Morrison et al 1982, Piiron et al 1986, Ko et al 2003). Engelsen and Hansen (2009) determined tocopherol content of roller milled wheat flour fractions. The authors reported 44.6 µg/g of total tocol content in whole wheat flour, 286 µg/g of total tocol content in germ fraction and 40.1 and 82.8 µg/g tocol content in coarse and fine bran fractions respectively. Engelsen and Hansen (2009) concluded that the tocopherols were highly concentrated in wheat germ and tocotrienol was more equally distributed throughout the grain, with slightly higher content in outer bran fractions.

The free hydroxyl group on the aromatic ring in tocopherol is responsible for its antioxidant properties. Vitamin E acts as a protector of lipids in biological membranes by acting as an antioxidant and free radical scavenger through a generally accepted autoxidation mechanism (Christen 2000).

2.2.1.3. Phytic acid

Phytic acid is composed of a simple sugar (inositol) with six phosphate groups attached to each carbon. It is also known as inositol hexaphosphate (IP6) and is typically found in food sources high in fibre content (Shamsuddin 2002, Somasundar et al 2005). Phytate refer to the magnesium or calcium salt of IP6 and represents the main storage form of
phosphorus in cereal grains. IP6 forms complexes with divalent metals and is remarkably stable (Blaabjerg et al 2010). Phytic acid adopts different conformations depending on pH, and is sterically stable.

Interestingly, phytic acid likely represents the most highly concentrated phytochemical in wheat and wheat bran particular. Whole wheat contains 0.66-1.22% (dry weight) of phytic acid while wheat bran contains 4.59-5.52% (dry weight) (Lolas et al 1976). Accordingly, wheat bran is highly enriched in phytic acid compared to whole wheat grain (Lolas et al 1976). By contrast endosperm tissue of wheat contains very little phytic acid. Manthey (2002) also reported that 50% to 60% of total wheat phytic acid is found in durum wheat bran while only 4-6% was in found in semolina, confirming the above statement.

Phytic acid has traditionally been considered to be an antinutrient (most notably iron deficiency anemia) associated with its ability to bind divalent cations and reduce their bioavailability. However, a few studies have noted its antioxidant activity (Graf et al 1987, Graf and Eaton 1990, Martinez-Tome et al 2004) and many studies have indicated its efficacy to lower the risk of heart disease, diabetes and cancer especially, and numerous reviews on its preventive and possible therapeutic value have been published (Zhou and Erdman 1995, Fox and Eberl 2002, Jenab and Thomson 2002, Singh and Agarwal 2005, Vucenik and Shamsuddin 2006, Kumar et al 2010).

2.3. Antioxidant activity of wheat bran components

Phytochemicals (phenolic acids, carotenoids, tocopherols, tocotrenols, and flavonoids) provide the majority of the antioxidant activities of foods (Tsao 2008). Antioxidants are a group of small molecular weight phytochemicals (as described in previous
sections) present in wheat grain. In wheat, most of these phytochemicals are found in the bran fraction (Tsao 2008). Antioxidant activity is an important biological property of many phytochemicals that protects living organisms from oxidative stress. It is believed that, phenolic compounds have relatively very strong antioxidant activities. The majority of phytochemicals present in wheat bran are phenolic compounds which make up more than 60% of the total (Table 2.3). It has been postulated that for tissues and organs under severe oxidative stress, there is excessive formation of reactive oxygen species, most notably free radicals, which can damage biomolecules such as DNA, proteins, membrane lipids and carbohydrates and this can lead to several disease conditions (Halliwell 1996). Oxidative damage is a major contributor in the development of chronic diseases such as atherosclerosis (Minhajuddin et al 2005). Elevated levels of oxidants such as superoxide anion, hydrogen peroxide or nitric oxide may damage tissues by direct oxidation of key biological molecules and alteration of transcription factors (Bowers et al 2004). Antioxidant may modulate this oxidative stress and prevent the biologically important molecules from oxidative damage, therefore reducing the risk of several chronic diseases including cardiovascular diseases. Native low density lipoprotein (LDL) is not atherogenic, whereas oxidized LDL considered to be highly atherogenic and toxic to vascular cells (Rota et al 1998). One potential mechanism proposed in the beneficial action of antioxidants in food systems is that they can directly quench free radicals to terminate the radical chain reaction and prevent or lower the extent of LDL oxidation (Zhou et al 2007).

Both oxidants and antioxidants have different characteristics. Individual antioxidants may act by multiple mechanisms in a single system or by different single mechanisms depending on the reaction system. Antioxidants respond in different ways to different radical
or oxidant sources (Wright et al 2001). Free radical scavenging activity of phytochemicals in wheat and wheat bran vary according to wheat variety, location and environmental conditions (Yu et al 2002). Antioxidant activity estimation is highly affected by the reactive oxygen species presented in the assay. While one antioxidant may be extremely effective in scavenging one type of free radical, it can be relatively ineffective towards another type of free radical (Moore and Yu 2008b). Therefore there is no single assay that can accurately measure all free radical sources or all antioxidants in a mixed or complex system. It is better to use more than one method to obtain accurate result of antioxidant activities. But too many analytical methods can result in inconsistent readings, incorrect interpretation of assays and improper specification of AOA (Schlesier et al 2002, Prior et al 2005).

2.3.1. Milling fractions and wheat antioxidant activity

Liyana-Pathirana and Shahidi (2006) reported higher levels of total antioxidant capacity in wheat bran (10-14 mmol Trolox equivalents [TE]/g) compared to whole wheat (4-5 mmol TE/g) using extracts composed of 80% aqueous ethanol. The authors suggested that higher levels of antioxidant activity in wheat bran was mainly due to their high levels of total phenolic acid content (2500-3500 mg Fe equivalents/g) compared to whole wheat grain (800-1500 mg Fe equivalent/g). Wheat phenolic compounds are concentrated in cell wall materials in the bran fraction (Fulcher et al 1996). Higher levels of phenolics are typically associated with higher levels of antioxidant capacity in wheat bran or other material. Liyana-Pathirana and Shahidi (2007) evaluated whole wheat, flour, bran and shorts for their AOA using 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging methods. Shorts exhibited higher levels of AOA (63.4 - 69.8 µmol TE/g) followed by wheat bran (51.9 - 55.8 µmol TE/g), whole grain (44.7-47.5 µmol TE/g) and flour (25.3 - 27.1 µmol TE/g). Even though germ exhibited high levels of AOA, wheat germ represents only 3% of whole kernel weight, so its contribution to total AOA in whole wheat is much less compared to wheat bran, which represents 14-15% of whole kernel weight. Liyana-Pathirana et al (2006) also determined AOA of wheat in pearling fractions of 10-50% of the whole kernel. Not surprisingly, AOA decreased in pearled wheat with progressive processing, and the initial or 10% pearling by-product, by weight, had the highest AOA, presumably because it contained the highest concentration of bran including aleurone, and the lowest content of endosperm. Wang et al (2008) similarly studied AOA of wheat pearling fractions and obtained results similar to those of Liyana-Pathirana and Shahidi (2007). They also reported increasing AOA with pentosan content in
wheat pearlings. Hung et al (2009) similarly used a pearling technique applied to waxy wheat grain (3% amylose) and measured phenolic and flavonoid contents and AOA of free and bound phenolic fractions adjusted from the method of Sosulski et al (1982). As with previous studies, phenolic content and AOA increased in a correlated manner from the innermost to outermost layers of the wheat grain. Beta et al (2005) conducted a study to evaluate AOA of wheat milling fractions resulting from pearling. In this study, wheat grain was pearled more precisely compared to other similar studies and five different pearling fractions (5%, 10%, 15%, 20% and 25%) in 5% increments of whole grain by weight. In contrast to other studies the outermost pearling fraction (5%) did not possess the highest AOA, which was the property of the next incremental pearling fraction.

2.3.2. Distribution of antioxidants in wheat bran

Antioxidants in wheat are closely associated with the bran tissue and concentration of constituent phenolic compounds which can be fractionated as free, soluble conjugated (or esterified) and insoluble bound forms (Krygier et al 1982, Liu et al 2005, Shahidi et al 2006, Hung et al 2009). As indicated by papers cited above, phenolic compounds are concentrated in the outermost portions of the wheat kernel, especially in aleurone layer of bran (Antoine et al 2005, Parker et al 2005). A reported 75% of total phenolics were in bound form, contributing 90% to the total antioxidant activity (Sosulski et al 1982, Liyana-Pathirana and Shahidi 2006). Verma et al (2008) studied AOA of wheat bran of over 50 genotypes using DPPH and ABTS free radical scavenging methods. The authors found approximately three times the level of bound phenolics compared to free phenolics and a similar distribution of AOA. Cui et al (2006) has also reported high levels of AOA for bound fraction compared to
free phenolics. Liyana-Pathirana and Shahidi (2006) carried out a detailed study on different fractions of antioxidants present in wheat bran. They evaluated AOA in extracts corresponding to free, esterified and bound phenolics. Trolox Equivalent Antioxidant Capacity (TEAC) assay and DPPH radical scavenging assays were used to measure AOA of these fractions. AOA of the free fraction was 13.4-14.2 µmol TE/g defatted material. The corresponding AOA in the esterified fraction was 29.1-33.5 µmol TE/g defatted material. In contrast, the AOA of wheat bran in the bound fraction was approximately ten times higher (237-269 µmol TE/g defatted material). This indicated that most of the antioxidants present in wheat bran were in bound form. It is important to release bound phenolics from cell walls to evaluate total antioxidant capacity of wheat bran. Similar levels of AOA of free and bound fractions were reported by Serpen et al (2008).

2.3.3. Variables that can affect antioxidant activity of wheat bran

Various properties of an antioxidant assay system can greatly affect measurement outcomes. There is at the outset variation caused by basic differences in the chemical mechanisms of an antioxidant test which include metal chelation, free radical scavenging, and oxidative enzyme inducers or inhibitors and their interactions amongst each other and physicochemical factors of the assay system, such as pH and viscosity. The most obvious sources of variation include the concentration and type of antioxidants present in a sample, e.g. phenolic acids vs. flavonoids, as well as extraction solvent type, solvent polarity and concentration, sample-to-solvent ratio, extraction time and temperature. The nature and history of the sample is also an important source of variation, e.g. is it a powder or a pellet, or has the sample been preprocessed hydrothermally like many ready-to-eat breakfast cereals?
In that regard, matrix effects, as covered in the previous section and commented upon further below, can create extraction differences and issues, e.g. are the antioxidants freely extractable or contained or bound within cells or cell walls heavily laden with non-starch polysaccharides and lignin.

There are many methods to evaluate AOA of wheat based products, and these have been comprehensively reviewed (Moore and Yu 2008a). These include scavenging assays for various free radicals such as DPPH, ABTS cation, superoxide anion, and hydroxyl radical test for hydrophilic and hydrophobic antioxidants. Additional tests include measuring oxygen radical absorbing capacity (ORAC), total phenolic content, iron chelating capacity, copper chelating capacity, lipid peroxidation inhibition, and low density lipoprotein peroxidation inhibition assay. All these different assays produce different results in terms of AOA of wheat and wheat bran (Yu and Cheng 2008). However, all those different results are typically correlated (Moore and Yu 2008a). A few studies have reported antioxidant activity of specific wheat phenolic acids (Onyenho et al 1992, Rice-Evans 1996, Galato et al 2001, Zhou et al 2005, Kim et al 2006, Zhou et al 2006, Yu and Cheng 2008) as well as AOA of phytic acid (Graf and Eaton 1990, Graf et al 1987, Martinez-Tome et al 2004). Wheat phenolic acids can show very different levels of antioxidant activity for different antioxidant methods (Yu and Cheng 2008). Among the several phenolic acids that are present in wheat (in descending order of concentration: ferulic, syringic, vanillic and p-coumaric), ferulic acid, which is predominant, tends to show relatively intermediate to high levels of AOA depending on the specific AOA method.

Bran form and history as noted above can affect AOA results. Martinez-Tome et al (2004) evaluated antioxidant capacity of commercial samples of oat and wheat bran in
different forms, e.g. crunchy oat bran, oat bran alone, oat breakfast cereal, wheat bran alone, wheat bran powder, wheat bran tablet, wheat bran with malt flavour and a bran breakfast cereal. No clear descriptions were provided for these samples, e.g. the difference between “wheat bran alone” and wheat bran powder was not described. Presumably the difference was particle size. Bran samples were extracted with water by agitating for 30 min. Frozen supernatants were studied using various different antioxidant activity assays including peroxidation of phospholipid liposomes, hydroxyl radical scavenging, scavenging of hydrogen peroxide, total AOA by the TEAC assay (essentially an ABTS radical scavenging assay), the Rancimat test (for oxidative stability of butter), and inhibition of linoleic acid peroxidation. Among the different wheat bran products, wheat bran powder typically had the highest AOA values, followed by wheat bran alone. In contrast, bran tablets had much lower AOA, presumably due to its compressed form limiting water extractability. In general, this study clearly indicated varying results of AOA of wheat bran and products containing wheat bran by different antioxidant assays possibly due to extraction efficacy with water and reaction differences among the different assays. Furthermore, compared to oat bran in different forms, wheat bran showed considerably higher antioxidant capacities by free radical scavenging assays. However, the processed oat brans (crunchy oat bran and oat breakfast cereal), but not oat bran alone, had higher Rancimat test values compared to wheat bran indicating greater protection against accelerated oxidation.

2.3.4. Effect of extraction solvents and extraction methods

The effects of extraction methods on AOA of wheat and wheat bran have been well reviewed (Moore and Yu 2008b). The most popular solvents used for extraction include
methanol, ethanol, acetone either alone and/or diluted with water (e.g. 50% acetone or 80% ethanol or 80% ethanol) and/or in combinations (e.g. methanol/acetone/water, 7:7:6, v/v/v) or with acid (e.g. acidified methanol). Simple batch solvent extraction at ambient temperature and neutral pH for several hours has been the most commonly used extraction method for wheat antioxidants, while a few studies have reported using Soxhlet-type reflux extraction at elevated temperatures.

Mageed and Fadel (1999) extracted antioxidants from wheat bran with hexane, chloroform, and ethanol using simple continuous extraction for 12 h at a solid-to-solvent ratio of 1:5 (w/v). These extracts were then compared for their capacities in suppressing lipid peroxidation in cooked beef. Results showed ethanol to have the highest extraction yield at 1.7% (w/w) followed by hexane and chloroform at 1.5% and 1.06% (w/w), respectively. Results also showed ethanol extracts to be more effective than both hexane and chloroform extracts at inhibiting lipid oxidation during 7 days of meat storage. Similarly, Krings et al (2000) compared ethanol, acetone, and diethyl ether extracts (16 h at a solid-to-solvent ratio of 1:8 (w/v)) of roasted wheat germ for inhibition against lipid oxidation in stripped corn oil over 10 days. Similar to Mageed and Fadel (1999) ethanol (more polar than acetone or diethyl ether) was found to be the most effective solvent for extracting lipid soluble antioxidants capable of inhibiting lipid oxidation. Oufnac et al (2007) also studied pure organic solvents (methanol, hexane and acetone) on the extraction of total phenolic compounds and DPPH free radical scavenging AOA of wheat bran. Extraction time was 12 h at 60 °C using a sample-to-solvent ratio of 1:4 (w/v). As in previous studies, the more polar solvent, in this case methanol, extracted more phenolics (by ~ 250%) and those extracts had
considerably higher AOA compared to the other solvents; more than twice that of acetone which was itself significantly higher than that of hexane.

Much shorter extraction conditions (30 min) were evaluated by Zielinski and Kozlowska (2000) who compared water (at 4 °C) and 80% methanol (20 °C) for extraction of whole wheat of two genotypes (1:10 sample-to-solvent ratio, w/v) on total phenolic content and ABTS radical scavenging AOA. Whereas phenolic content (by Folin-Ciocalteu method) of the 80% methanol extracts was on average ~8% more than that of water extracts, AOA was almost 350% higher for the methanolic extracts when calculated per unit weight of whole grain (Moore and Yu 2008a). While these results cannot be compared with earlier studies, they do indicate the superior efficiency of 80% methanol over pure water for extraction of free radical scavenging compounds of wheat grain.

The aforementioned studies beg the question of whether pure or diluted solvent extracts of whole wheat or wheat bran are different in AOA. That question was partially answered by Zhou and Yu (2004) who compared 50% acetone, 100% ethanol, 70% ethanol, and 70% methanol to extract wheat bran of two genotypes for total phenolic content and free radical AOA using DPPH, ABTS and ORAC methods. Extraction conditions were 15 h at ambient temperature with a sample-to-solvent ratio of 1:10 (w/v). Extraction efficiency for phenolics was in the order 50% acetone > 70% methanol > 70% ethanol > ethanol, with 50% acetone extracting more than 3.6 times the phenolic yield of ethanol. Likewise for the AOA assays, 100% ethanol was the least effective solvent to extract antioxidants. For the other solvents, extraction efficiency for AOA depended on the AOA test, with 50% acetone extracts showing the highest scavenging capacity against ABTS, while 70% ethanol had slightly higher ORAC values than 50% acetone extracts, and 70% methanol was the most
effective solvent for extracting DPPH scavenging compounds from wheat bran. The authors concluded that based on results for total phenolic content and AOA, 50% acetone was the preferred solvent to prepare antioxidants extracts from wheat bran for routine analytical work.

Somewhat different results were reported in a related study (Moore and Yu 2008b) where in contrast to wheat bran, ground whole grains of eight soft wheat genotypes were extracted with 50% acetone, 80% methanol and 100% ethanol and extracts were evaluated for phenolic content and AOA by three radical scavenging methods (ABTS, ORAC and superoxide) using similar conditions as described by Zhou and Yu (2004). As with the latter study, Moore and Yu (2008b) found that 50% acetone was the most effective extraction solvent for estimating ABTS radical scavenging capacity. 50% acetone was also the most effective extraction solvent for estimating ORAC values which were more than twice that obtained by using 80% methanol as a solvent; ORAC values were very low using 100% ethanol. Surprisingly and unlike results reported by Zhou and Yu (2004), 50% acetone was the least effective solvent for extracting phenolic compounds from the ground whole wheat samples and was also relatively inefficient to extract superoxide radical scavengers in which case 80% methanol was the best solvent.

Onofre and Hettiarachchy (2007) compared water, methanol, and 40, 60, and 80% ethanol solutions (v/v) to extract phenolic acids from ground rice bran (400 mg to 10 mL solvent) that was briefly sonicated. Water and 60% ethanol extracted virtually the same amount of phenolics from rice bran, although it was not statistically different from the total phenolics extracted by 80% ethanol. An increase in the ethanol content from 40 to 60%
significantly increased the total phenolics extracted, although an increase from 60 to 80% did not produce a further increase.

These results suggest strongly that extraction solvents for antioxidant properties need to be optimized for each type of wheat material analyzed as well as for the specific antioxidant property measured. The reasons are that each type of wheat material or fraction is likely to have different phenolic and antioxidant compositions and that even matrix effects will influence extractions. For example, wheat fractions enriched or deficient in cell walls containing varying content and composition of non-starch polysaccharides are likely to resist extraction using simply organic solvents in varying degrees. Needless to say, these results present serious complications for a comprehensive understanding of the nature of wheat antioxidant activity.

2.3.5. Genotype and environment effects on wheat bran antioxidant activity

Verma et al (2008) studied phenolic content and antioxidant properties of bran in 51 wheat cultivars representing nine wheat classes including spelt wheat and triticale. The authors reported a total phenolic content range from 4.6-6.7 mg/g gallic acid equivalent of bran. AOA of wheat bran ranged from 11.9% to 20.1% using a DPPH radical decolourization assay applied to methanolic extracts. The authors concluded that AOA varied significantly among wheat classes but variation was greater among individual wheat cultivars in certain classes. Furthermore, this study showed weak correlation between AOA and total phenolic content in wheat bran \((r =0.47)\), but that correlation improved \((r=0.80)\) when the results were taken as wheat class averages. Results supported the observation that wheat genotype or growing location can have a significant influence on whole wheat antioxidant activity (Yu et
al 2002, 2003, Zhou 2004, Mpofu et al 2006). However, it should be noted that the comprehensive results reported by Verma et al (2008) describe only a modest level of AOA variation by genotype as evidenced by coefficient of variation of ~10% for AOA of methanol extracts.

2.4. Treatments of wheat bran to enhance properties

Whole wheat and bran contain many phytochemicals as well as fibre that are mainly present in the bound form, i.e. covalently linked to or contained within cell wall structural components such as cellulose, lignin, and proteins. Ferulic acid for example is mainly covalently conjugated to mono and disaccharides, cell wall polysaccharides, glycoproteins, polyamines and lignin. Food processing operations such as particle size reduction, hydration and thermal and/or pressure treatments can contribute to the release of bound phenolic acids (Dewanto et al 2002). The following section reviews the effects of wheat bran treatments to improve the functional and nutritional quality of wheat bran.

2.4.1. Reducing bran particle size

Loaf volume of whole wheat bread has been negatively correlated to bran particle size. Varying granulation by sifting gives a different composition in particle fractions than that obtainable by simple grinding. It has been reported that finely ground bran particles (0.5 mm) yield lower loaf volumes than that for coarse bran (Galliard and Gallagher 1988). Bran particle size also plays a role related to whole wheat bread quality. However, results obtained from different studies are often contradictory (Lai et al 1989b, Galliard and Gallagher 1988, Zhang and Moore 1999).
Zhang and Moore (1999) and de Kock et al (1999) studied the effect of wheat bran particle size to improve whole wheat bread quality. Zhang et al (1999) reported improved bread quality by increasing bread volume achieved using fine bran particle sizes (415 µm). Reducing bread loaf volume is one of the negative side effects that has been reported when wheat bran is added to a bread dough mixture. There have been no published reports on the effects of particle size on antioxidant activity of wheat bran.

According to Wooten et al (1986) bran was extracted with water prior to use in a dough mix. After discarding the water extracts the residue bran was added to a bread formulation. This resulted in higher loaf volume bread when coarse bran particles were added compared to fine bran particles. It was concluded that washing out of some unidentified chemical compounds in bran resulted in increased loaf volume of bread.

2.4.2. Hydration of bran

Lai et al (1989b) reported that “soaking” bran with water prior to its addition to a bread formulation resulted in improved loaf volume. The authors hydrated 14 g of fine and coarse bran flour in water (21, 31 and 35 mL) and added soaked bran on a replacement basis to a 100 g flour dough formula. Significantly higher loaf volumes were obtained when the bran was added wet than in the dry state. Moreover, fine hydrated bran had significantly higher loaf volume compared to coarse hydrated bran. The authors did not provide an explanation for the improvement in loaf volume observed.

Nelles et al (1998) reported positive significant effects of pre-treating bran including hydration and so-called “wet heat and wet oxidation” on dough mixing and whole wheat bread quality. In the hydration experiment, bran (144 g) was suspended in excess water at 10
°C for 15 minutes, and then wrung out to achieve a constant weight (500 g). For wet oxidation the same method was employed except that the bran was suspended in oxygenated water. For wet heating, again the same method was used except bran was suspended in boiling water before being wrung out to constant bran weight. Hydration of bran significantly improved whole wheat bread height and volume (by ~10%) and also reduced bread firmness by 27%. Heat treated bran (at 121 °C) was also evaluated for oxygen uptake compared to untreated bran. Heat treated bran absorbed substantially less oxygen presumably due to deactivation of lipoxygenase and other heat labile enzymes such as lipase thereby decreasing potentially oxidizable substances in the bran (Galliard et al 1986). Overall, the hydration and wet oxidation treatments improved whole wheat bread quality significantly more than the wet heat treatment.

2.4.3. Fermentation of bran

Increasing amount of fibre, i.e. soluble and insoluble cell wall material, has detrimental effects on dough structure of the whole wheat bread. Different treatments as noted above have been applied to bran to improve hydration and/or inactivate enzymes to improve the quality of the whole wheat bread. Fermentation of the bran fraction using yeast and Lactobacillus brevis (lactic acid bacteria) has also been studied to improve breadmaking quality and loaf volume specifically (Salmenkallio-Marttila et al 2001). Bran pre-treated with yeast and lactic acid bacteria, in short and long fermentation improved bread quality (specific bread volume) over untreated bran by 10-15%. Wheat bran at a substitution level of 20% decreased loaf volume by 19% in comparison to white wheat bread. However fermentation of bran reduced this difference to about 7% and increased the loaf volume
significantly compared to adding untreated bran in the whole wheat bread formula. Taste and mouthfeel of the whole wheat bread also improved due to the fermentation pre-treatment (Salmenkallio-Marttila et al 2001).

2.4.4. Chemical treatment of wheat bran to enhance extractability

Baublis et al (2000) studied the antioxidant activity of breakfast cereals. The authors reported that esterified phenolic acids had somewhat higher antioxidant capacity compared to free phenolic acids based on a lipid oxidation assay. Baublis et al (2000) also reported that compared to simple aqueous extracts, simulated gastrointestinal pH conditions caused a substantial increase in antioxidant activity (reduction in liposome oxidation) for the high bran and whole grain cereal aqueous extracts and their low molecular weight fractions. The authors suggested that acid conditions caused changes in the activity, composition and/or concentration of water- extractable antioxidants.

The extent to which phenolic antioxidant compounds can be liberated from whole grains by chemical treatment was compellingly revealed by standard procedures to solubilize insoluble bound phenolics. For example Sosulski et al (1982) treated cereals (wheat, rice, corn, oat) with 4M NaOH and acidified suspensions to pH 2 to extract bound and esterified phenolics. This resulted in substantially higher yields of total phenolics (15 times). Krygier et al (1982) treated rapeseed meal in the same way and reported 10-15 times higher total phenolic levels. Liyana-Pathirana and Shahidi (2006) used similar condition for wheat bran and likewise obtained 15 times the concentration of total phenolic compared to free phenolic levels available before NaOH treatment.
2.4.5. Heat treatments of bran and other grain products

There have been very few studies done on the chemical composition of wheat bran after heat treatment. Few studies in the past have subjected bran to different types of heat treatments to obtain acceptable whole wheat breads and possibly increase nutrient availability. Caprez et al (1986) heat treated wheat bran in different ways and its chemical and physical composition was examined. Heat treatment included boiling, steam cooking, autoclaving, roasting, micronizing and extrusion. Compared to untreated ground wheat bran (< 0.4 mm), boiling (15 min in 10-fold water at 100 °C) and autoclaving (15 min at 121 °C and 2 bar) resulted in significant increase in total dietary fibre analysed likely due to the concomitant significant increase in soluble fibre from 7.8% (untreated bran) to 11.2 and 11.7% for boiled and autoclaved bran, respectively. The autoclaved wheat bran also showed the largest differences in farinograph properties of flour-bran mixtures comprising 80% patent flour and 20% bran; compared to untreated bran, maximum resistance declined by 25% suggesting weakened whole wheat doughs, but mixing time increased by 30%, and mixing tolerance increased substantially indicating stronger doughs. It was not clear whether the authors adjusted water absorption in the farinograph to account for a possible decrease in moisture content of the bran after autoclaving.

According to de Kock et al (1999) heat treating bran (autoclaved for 1.5 hours at 121 °C in sealed vessels, i.e. no pressure effect), depending on the bran particle size and milling origin, resulted in higher loaf volumes and loaf heights of whole wheat bread incorporating 12% bran (in proportion to flour) of intermediate particle size (> 0.75 mm, < 1.8 mm). This bran sizing and a larger particle size was described as originating from the outermost layer of bran, whereas bran of smaller particle size was described as originating from the innermost
layer. The increases in loaf volume and height were in the order of 7% and 5%, respectively compared to unheated bran of the same particle size. Bran of lower or higher particle size when heated produced no significant differences in baking quality. Heating of the wheat bran resulted in considerable reduction in total reducing substances (presumed to be glutathione), by ~30% and lipase activity by ~90%. Nelles et al (1998) reported similar effects on lipase activity using a wet heat treatment. The authors (de Kock et al 1999) surmised that heat-labile chemical substances in bran were in part responsible for depression of loaf volume.

Izydorczyk et al (2000) studied the effects of various heat treatments of barley grain on extractability and molecular characteristics of soluble β-glucan. Hydrothermal treatments of autoclaving (121 °C, 2 PSI for 30 min) and steaming (20 min over boiling water) had no effect on extractability of β-glucan, but prevented enzymic hydrolysis, and thereby substantially improved β-glucan molecular weight and viscosity development of barley slurries in a Bohlin rheometer. The samples obtained much higher maximum viscosity values compared to untreated control samples and no detectable decline in viscosity with time indicating no presence of β-glucanase activity. In contrast, roasting of barley grain at 105 °C for 30 min produced results very similar to untreated barley; there was little to no increase in viscosity development.

de Carvalho et al (1996) subjected rice bran to oven heat treatment (105 °C, 3 h) and autoclave heat treatment (125 °C, 20 min, 7456 Pa.) and then blended with wheat flour to evaluate rheological properties. Heat treatment increased pasta viscosity and retrogradation rate and weakened the dough. However heated bran did not increase the water absorption of the dough.
Saulnier et al (2001) destarched micronized maize bran (1.5 h in boiling water with thermostabile α-amylase, final yield = 82% dm) and the water insoluble fibrous residue (5 g in 50 mL water) was further heat treated by simulated flash explosion (160 °C to 210 °C, and 4 to 19 bar pressure, over 1 min, preceded by 15-25 min to raise the temperature and followed by pressure release and fast cooling) and autoclaving (160 °C for 0.5 to 4 h, no pressure specification). The extractability of arabinose, xylose and galactose (but not glucose) as well as ferulic acid increased substantially with flash explosion temperature; at 210 °C from 60-80% of the sugars and ~ 68% of total ferulic acid were solubilized. The authors reported that the ferulic acid ester linkage survived the conditions of pressure and temperature used, i.e. ferulic acid remained largely esterified to neutral sugars. For autoclaving, solubilisation of arabinose, xylose, galactose and total ferulic acid (~95% still esterified) reached a plateau value after 1 hour of treatment and up to 80-85% of the components were solubilized. Presumably, the concomitant increase in levels of solubilized ferulic acid together with arabinose and xylose arose from the molecular association of ferulic acid as a crosslinked component to arabinoxylan in bran (Wang et al 2006). Saulnier et al (2001) attributed the increase in solubility of the neutral sugars and ferulic acid upon flash explosion (and presumably also autoclaving) to autohydrolysis of cell wall polysaccharides (Excoffier et al 1991) and further structural breakdown caused by decompression (Debzi 1992). In this way cell walls in the bran are weakened and become more labile or accessible to enzymes and solvents in general.

Using experimental material somewhat similar to that used by Saulnier et al (2001), Bergmans et al (1996) prepared water-unextractable cell wall material (WUS) from wheat bran and proceeded to study the extraction of arabinoxylans by pre-treating the WUS in
distilled water in an autoclave autoclaved for 1 hr at 121 °C and 1 MPa over pressure, and subsequently by increasing the temperature and concentration of barium hydroxide as a selective solvent. The authors reported that by increasing the temperature of extraction, it was possible to improve the yield of arabinoxylans from 29% at 20 °C to 50% at 95 °C. However the autoclaving pre-treatment did not result in increased yield of arabinoxylans.

Acar et al (2009) subjected pulses, nuts and seeds to roasting (150 °C for 10, 30 and 60 min) and the antioxidant ABTS radical scavenging activity of extracts in an ethanolic-aqueous solution was determined. Pulses showed significantly increasing TEAC values (20%) with increasing towards increasing roasting time, whereas seeds had decreasing TEAC values (6%) with increasing roasting time. Nuts, depending on the species, had varying outcomes. The authors concluded that roasting had both positive and negative consequences in terms of the total antioxidant capacity in these food products and that the net effect depended on the balance between the thermal degradation of naturally occurring antioxidant compounds and the formation of new Maillard reaction by-products having antioxidant capacity.

Kadakal et al (2009) compared the effects on soluble vitamins of cooking (90 and 100 °C for ~ 90 min) wheat (1 part wheat to 15 parts water) and autoclaving of soaked wheat at 45% moisture content at 121 °C for 17 min. Content of thiamin, niacin, pantothenic acid pyridoxine, and riboflavin were all lower for the autoclaved soaked wheat compared to cooked counterparts. Compared to unheated wheat, thiamin, niacin, pantothenic acid, pyridoxine and riboflavin vitamin content were reduced by 37%, 59%, 33%, 65% and 44%, respectively.
Onofre and Hettiarachchy (2007) subjected rice bran to sonication at 25 °C for 1 min and then subjected water suspensions (1:100 sample to solvent ratio) by autoclaving (121 °C, 20 min) or heating in a water bath varying temperature (80 to 95 °C) and time (0.5 or 1 hr). Extracted phenolics increased from 7.08 mg/g in the optimum hot water bath condition (95 °C for 1 hr) to 8.43 mg/g by autoclaving. The authors hypothesized that the combination of both high pressure and temperature contributed to a more efficient extraction of phenolics from rice bran. The authors cited Cacace and Mazza (2003) who observed that during the extraction of phenolic compounds from milled berries, higher temperatures increased the rate of diffusion of total phenolic and anthocyanin molecules through the solid phase to the solvent (aqueous ethanol and sulfured water). They suggested that the increase in diffusivity due to temperature may be caused by an increase of the internal energy of the molecules and thus their mobility, and a reduction of the dynamic viscosity of the liquid phase.

Oufanc et al (2007) studied the effect of extraction of antioxidants in wheat bran through conventional solvent (methanol, acetone and hexane) extraction for 20 min at 60°C method and microwave assisted extraction using methanol only in sealed containers at elevated temperatures from 60 °C to 120 °C. Compared to conventional heating, microwave assisted heating produced increasing levels of extractable phenolics and antioxidant activity (DPPH free radical scavenging) with increasing temperature up to 120 °C. Compared to results at 60 °C, phenolic content and antioxidant activity of methanolic extracts at 120 °C were increased by ~100% and ~ 75%, respectively.

Inglett et al (2009) reported that distillers dried grains with solubles with high phenolic content and antioxidant activity can be obtained via extraction with water or 50% ethanol and high temperatures (100 or 150 °C) achieved in their study by the use of
microwave irradiation of suspensions sealed in a reactor vessel. In contrast Zadernowski et al (1999) reported that heat treatment of moistened (30% moisture content) ground oat grain (100 °C, 30 min) had no effect on total phenolic content of extracts, whereas extrusion at high temperature and pressure (~150 °C and 4 MPa.) resulted in 65% reduction in the amount of extractable phenolic compounds compared to unprocessed grain.

Bryngelsson et al (2002) applied different heating treatments (steaming, autoclaving (2.4 bar, 100-120 °C, 16 min), drum drying) to different forms of oats (groats, rolled, hulls) and evaluated methanolic extracts for content of tocopherols, tocotrienols, avenathramides, cinnamic acids (p-coumaric, ferulic, caffeic) and vanillin. The steaming treatment produced relatively small effects overall (compared to autoclaving – see below), although ferulic acid amounts in steamed (first of two steam treatment) groat extracts increased by ~35% compared to raw untreated groats, and increased again by a lesser percentage following a second steaming treatment and processing into rolled oats. In contrast, caffeic acid content decreased by ~ 50% following the initial steaming treatment, and vanillin content ~doubled following the second steaming treatment and processing into rolled oats. By comparison, autoclaving of grains, including the hulls, resulted in increased levels of \( \alpha \)- and \( \beta \)-tocopherol and \( \alpha \)-tocotrienol, whereas the increase in \( \beta \)-tocopherol was tremendous (4350%). Compared to steaming of groats, autoclaving of grains also had a much larger effect on levels of all cinnamic acids and vanillin; caffeic acid decreased to nondetectable levels, whereas the levels of ferulic acid, p-coumaric acid, and vanillin were greatly increased (222%, 1137%, and 1044%, respectively). In contrast autoclaving tended to reduce total levels of avenathramides. Drum drying largely decreased levels of most of the phytochemicals studied. Increases in p-coumaric acid and ferulic acid during the heating processes were
thought to arise from a release of these compounds from cell wall bound forms by hydrolysis. The authors suggested that the large increase in p-coumaric acid by autoclaving of grains was probably mainly due to release from the hulls because this cinnamic acid is part of Klason lignin, which amounts to about 22% of the oat hull but only 1.4% of the groats. The authors also stated that the higher levels in wholemeal compared to rolled oats also indicate that compounds released in hulls are transferred to the groats during processing. The preferential loss of caffeic acid in steamed and autoclaved samples was explained on the basis of reported differences in heat stability of phenolic acids; whereas caffeic acid is labile to heat in solutions at physiological pH, p-coumaric and ferulic acids are more stable (Dimberg et al 1996, Dimberg et al 2001). Also vanillin may be produced by the thermal decomposition of ferulic acid (Fiddler et al 1967). The decrease in all cinnamic acids and vanillin caused by drum drying may be due to oxidation, as it was indicated that some oxidation took place during this process. Bryngelsson et al (2002) concluded that is important to analyze the actual product, and not the raw material, to know the levels of antioxidants in the food product.

Regarding the subject of heat processing of grain and oxidation effects, Lehtinen et al (2003) studied the effect of heat treatment (steam and extrusion processing) on oat lipid oxidation which revealed an inverse relationship between residual lipase activity in whole kernels or bran-enriched flour and oxidation of lipids and production of volatile rancidity-related oxidation products during prolonged storage of the dry fractions. The authors found that if bran was heat treated to zero lipase activity, the amount of headspace hexanal detected after 12-month storage was 5 to 7 times larger than detected in non-heat treated bran. This formation of hexanal was linked to the oxidation of polar lipids. If the heat treatment was
totally omitted, the oxidation of unsaturated fatty acids in polar lipids did not occur even
during prolonged storage, although lipid hydrolysis to free fatty acids continued. The
oxidation of polar lipids suggested heat induced disintegration of membrane structures and
inactivation of heat labile antioxidants. The study indicated that heat treatment of
intrinsically high lipid containing oat products to minimize the production of free fatty acids
may inadvertently contribute to rancidity by promoting lipid oxidation which was found to
occur after 12 months even in both polar and storage lipids of bran enriched flour under
conditions of so-called “mild” heat treatment (90 °C for 20 min).

In contrast to most of the studies reviewed above which show modest to large
favourable improvements in properties of various grains or fractions due to thermal
treatments, Zhang et al (2010) reported that heat treatments applied to ground buckwheat
flour, like roasting, pressured-steam heating and microwave heating all caused a decline in
total phenolics, total flavonoids and antioxidant activity in acidified 70% acetone extracts,
with roasting producing the smallest effects. In general for all heat treatments, phenolic
content decreases were much less than those observed for antioxidant activities which were
assessed by hydroxyl and superoxide scavenging methods and lipid peroxidation inhibition.
Results reported by Zhang et al (2010) were in general agreement with an earlier study
(Sensoy et al 2006) who likewise evaluated buckwheat flour subjected to roasting (200 °C)
and extrusion (170 °C) heat treatments. In this study, total phenolic contents were not
affected by thermal processing, and antioxidant activity (DPPH radical scavenging) of
methanolic extracts declined by about 14%. Extrusion conditions did not cause any
significant changes.
In summary, different grains, fractions and products have been processed or treated in several different ways including chemical treatments, fermentation, enzyme hydrolysis, particle size reduction, hydration, and with heat. The research has focussed on effects on phenolic content, selected phytochemicals including phenolic acids, vitamins, or fibre solubility or on processing properties. However, there is essentially no research on the effect of heat treatment, particularly autoclaving which is an energy intensive process, on wheat or bran antioxidants and fibre constituents, especially considering those compounds or constituents that are extractable or soluble in water which was the main focus of this thesis research.
3. MATERIALS AND METHODS

3.1. Wheat bran samples

A representative sound sample (~ one tonne) of Canada Western Red Spring (CWRS) wheat was the principal material for the thesis research, and unless otherwise noted, CWRS bran was the basis for experimental results. In addition a sample of the U.S. Hard White Winter wheat cultivar Platte was also used for a few studies. Wheat was milled in the Canadian International Grains Institute’s pilot Buhler mill (milling capacity 474 kg/h). Flour, and resulting bran, was produced at an extraction rate of 76%. Coarse bran was collected as over tails of the final break roll of the mill; bran yield was approximately 11%. Coarse bran (>2-3 mm) was subsequently processed on a Jacobson model 120B hammer mill (Jacobson inc., Minneapolis, MN) to obtain two additional particle sizes. The bran was hammer milled to pass the following sieve openings: fine bran (<1.17 mm) and ultra-fine bran (< 0.586 mm). This was achieved by using different sieves against the hammers. Bran samples were stored at 4 °C for further analysis.

3.2. Thermal treatments

Bran, typically in 20 g amounts, was heat treated in a dry state (as is moisture basis) or moistened immediately prior to heat treatment. Wetting the bran involved using distilled water in a 2:1 ratio (w/v), i.e. 20 g bran and 10 mL water. The moistened sample was termed “wet bran”. Bran not similarly treated was termed “dry bran” or simply “bran”. Bran was subjected to treatments as specified in Table 3.1. A control sample refers to bran receiving no treatment.
Table 3.1. Description of bran heat treatments.

<table>
<thead>
<tr>
<th>Bran treatments</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclave</td>
<td>121 °C, 20 psig*, 15 min sterilization, 10 min drying.</td>
</tr>
<tr>
<td>Autoclave</td>
<td>132 °C, 22 psig, 15 min sterilization, 10 min drying.</td>
</tr>
<tr>
<td>Autoclave</td>
<td>121 °C, 20 psig, 15 – 90 min sterilization, no drying.</td>
</tr>
<tr>
<td>Autoclave</td>
<td>121 °C, 20 psig, 15 - 90 min sterilization, 10 min drying.</td>
</tr>
<tr>
<td>Autoclave</td>
<td>121 °C, 20 psig, 15 min sterilization, 10 min drying, 5 repeated cycles</td>
</tr>
<tr>
<td>Air oven</td>
<td>121 °C, atmospheric pressure, 15 min heating.</td>
</tr>
<tr>
<td>Air oven</td>
<td>121 °C, atmospheric pressure, 15 min heating, nitrogen flush</td>
</tr>
</tbody>
</table>

*psig = gauge pressure above atmospheric

One of the first treatments involved the type of containers in which bran was autoclaved, viz. a square bottom glass bottle, and in open aluminum pans. The glass bottles had dimensions 4 x 4 x 14 cm in height with a volume of 190 mL. The bottles were filled with bran (20 g) to a height of 5 cm which filled up ~ 90 mL of the bottle volume. The bottles were loosely capped for treatments. The aluminum pans had dimensions of 7 x 18 x 5 cm in height. Bran filled each aluminum pan to a depth of ~ 0.8 cm. The cross sectional area of the bran surface was ~ 126 cm². The samples in open pans were loosely covered with aluminum foil perforated with holes of ~3 mm diameter to prevent the bran from being moisten due to possible steam condensation in the autoclave upon decompression. These precautions were later deemed unnecessary due to the effectiveness of the exhaust and drying functions of the autoclave.

Samples were generally autoclaved at 121 °C with a 15 min sterilization time at 20 psig, i.e. 20 psi above atmosphere or at ~ 2 bar (AMSCO 3021, American sterilizer Co. Pittsburgh, PA). Accounting for pre-sterilization conditions and dying time (Table 3.2), the total basic autoclave time was about 31 min. Samples were also subjected to different sterilisation temperatures (121 °C and 132 °C) and different sterilization times (15 to 90 min)
with and without drying to evaluate antioxidant activity and color responses of wheat bran. Samples were also exposed to repeated autoclave cycles (1 to 5 cycles) using conditions specified in Table 3.2. Figure 3.1 shows the typical temperature and pressure profiles in an autoclave treatment cycle.

Table 3.2. The basic autoclave profile.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Phase</th>
<th>End temperature (°C)</th>
<th>Beginning pressure (psig)</th>
<th>End pressure (psig or mmHg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>Jacket charging</td>
<td>76</td>
<td>0.25</td>
<td>5.9</td>
</tr>
<tr>
<td>1-2</td>
<td>Purging</td>
<td>104</td>
<td>5.9</td>
<td>11</td>
</tr>
<tr>
<td>2-5</td>
<td>Charging to 121°C</td>
<td>121</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>5-20</td>
<td>Sterilizing</td>
<td>121</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>20-21</td>
<td>Exhaust and vacuum dry</td>
<td>94.6</td>
<td>5.4</td>
<td>8.6</td>
</tr>
<tr>
<td>21-31</td>
<td>Drying</td>
<td>85.2</td>
<td>9</td>
<td>5.4</td>
</tr>
</tbody>
</table>

*For drying steps pressure is expressed as mm Hg to reflect partial vacuum conditions

To compare basic autoclaving with heating of bran at atmospheric pressure, samples were air-oven treated in aluminum pans as described above, at 121°C for 15 min in a Fisher Scientific Isotemp model 496 controlled atmosphere moisture oven. For some experiments, the atmosphere inside the oven was flushed with nitrogen gas to exclude oxygen during heat treatment. Unless otherwise indicated the N₂ flow was 10 L/min.

Fine bran samples were subjected to superheated steam at atmospheric pressure according to a method described by Pronyk et al (2004). The superheated steam treatment system consisted of a steam generator, steam conveying pipelines, a drying chamber, auxiliary heaters (super heaters and heating tapes), a hot-air supply system, and a data
acquisition and control system. In the superheated steam experiment, two different temperatures (121 and 132°C) was tested as described in Table 3.1.

Figure 3.1. Temperature and gauge pressure profiles for a standard autoclave cycle.
3.2.1. Bran treatment nomenclature

Table 3.3 summarizes the nomenclature of the various brans and extracts according to the applied bran treatment.

<table>
<thead>
<tr>
<th>Bran treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Untreated bran</td>
</tr>
<tr>
<td>Autoclave dry</td>
<td>Autoclaved dry bran</td>
</tr>
<tr>
<td>Autoclave wet</td>
<td>Autoclaved moistened bran</td>
</tr>
<tr>
<td>Oven-air dry</td>
<td>Oven heated dry bran at ambient air atmosphere</td>
</tr>
<tr>
<td>Oven-air wet</td>
<td>Oven heated moistened bran at ambient air atmosphere</td>
</tr>
<tr>
<td>Oven-N dry</td>
<td>Oven heated dry bran in N₂ atmosphere</td>
</tr>
<tr>
<td>Oven-N wet</td>
<td>Oven heated moistened bran in N₂ atmosphere</td>
</tr>
</tbody>
</table>

3.3. Sample extraction for antioxidant activity measurement

Bran (20 g) was mixed with 300 mL distilled water (1:15 ratio w/v) in a 500 mL Erlenmeyer flask and was agitated using a magnetic stirrer. Unless otherwise noted, bran samples were extracted for 18 h at room temperature. In a separate experiment, the effect of extraction time (1 to 36 h) and temperature (4 °C, room temperature [~20 °C], 50 °C) was determined. Bran water suspensions were subsequently filtered using four layers of cotton fabric cheese cloth (Fisher Scientific, Cat No. 06-665-29) to remove bran particles. This was done to facilitate satisfactory subsequent centrifugation of the bran-water mixtures in order to obtain a particle–free supernatant extracts for analysis. The cheese cloth was tightly squeezed to extract filtrate. In the absence of this filtration treatment, centrifuged bran-water
suspensions did not form a solid pellet, presumably because the density of hydrated bran became close to that of water. Yield of the filtrate was ~ 250 mL.

The filtered extracts were centrifuged at 10240 x g (20 °C) for 10 min using a GSA rotor and RC5C Sorvall centrifuge (DuPont Co. Newtown, CT). Supernatants and fibrous residues were placed in plastic trays (cross-sectional area of 175.5 cm²), frozen at -20 °C, and freeze-dried (Unitop 600L, Virtis Co., Gardiner, NY). Freeze drying duration was ~ 7 days. Freeze-dried extracts and fibrous residues were ground using a blade coffee grinder to pass through a 500 µm sieve. Resulting material was stored in sealed plastic containers and frozen at -15 °C to -20 °C until analysis. Yield of the freeze-dried extracts was 10 – 12% of initial bran weight. The theoretical yield of the freeze-dried extracts was ~ 12-14% of initial bran weight taking in to account the ~ 17% loss of wet extract to the cheeses cloth before centrifugation. These freeze-dried water soluble extracts are henceforth referred to as “bran extracts”.

3.4. Dough mixing experiments using the Mixograph

Five different experiments were performed to evaluate bran, water-soluble extracts and/or residues on dough mixing behaviour using a computerized direct drive 2 g, or computerized 10 g moving bowl mixograph. The 2 g mixer was used to evaluate bran extracts that were available in lesser amounts. The mixograph mixes doughs at relatively high levels of intensity using pins and a planetary motion. This type of recording dough mixer is very well recognized and widely used, particularly in North America, for evaluating dough strength and mixing properties. A common refined CWRS wheat base flour (75% flour extraction) containing 2% salt (flour basis) was used as base flour for all dough mixing
experiments. Water absorption was held constant at 60%, and 88 rpm mixing speed was used. A spring setting of 12 was used with the 10 g mixograph. The following list outlines the dough mixing experiments.

- Effects of particle size of untreated bran; 10 g mixograph was used; 1.5 g of bran was added to 10 g of base flour. Accordingly, level of bran addition was 15%.
- Effects of autoclave-treated bran and extracts; 2 g mixograph was used. To 2 g of base flour, test materials were added in 2%, 5% and 10% levels for all three particle sizes.
- Comparison of autoclaved and oven treated fine bran; 2 g mixograph was used; bran, extracts and residues were tested at addition levels of 2%, 5%, 10% and 15% levels to the base flour (2 g).
- Effects of extraction temperature (4 °C, 20 °C and 50 °C) of autoclaved bran of three particles sizes; 2 g mixograph was used; (bran, extracts and residues were tested at additional levels of 2%, 5%, 10% and 15% to the base flour).
- Autoclaving of fine bran in bottles vs. pans (see page 45); 2 g mixograph was used; bran, extracts and residues were tested at levels of 2%, 5% and 10% to the base flour.

3.5 Determination of antioxidant activity

Antioxidant activity was determined by using two different free radical scavenging methods. Free radicals used were DPPH and ABTS. These antioxidant activity estimation methods are widely used to evaluate antioxidant compounds in foods or to quantify antioxidant activity in complex biological systems (Moore and Yu, 2008b). They are rapid, straightforward to carry out, and relatively inexpensive. Antioxidants can function in
different ways in both food and biological systems; hence, physiochemical properties of assay systems, such as viscosity, pH and selection of organic solvent can greatly influence effectiveness of antioxidants (Moore and Yu 2008b). It is therefore recommended to employ more than one antioxidant assay to determine antioxidant activity. However, results, at least as applied to wheat samples, appear to be highly correlated across many methods of AOA determinations (Liyana-Pathirana and Shahidi 2005, 2006, Iqbal et al 2007, Serpen et al 2007, 2008, Verma et al 2008).

Both DPPH and ABTS AOA methods are decolourization assays that measure the capacity of antioxidants to directly scavenge the radicals based on colour reduction at certain wavelengths as a function of time. Typically, the greater the capacity of a food extracts for free radical scavenging, the greater the degree of decolourization. A DPPH solution will change its color from dark blue to colourless, while an ABTS counterpart will change its colour from dark green to colourless when they convert from a radical form to non-radical form by antioxidants (Moore and Yu 2008b).

3.5.1. DPPH free radical scavenging assay

Preliminary experiments used 100% methanol as the extractant (1 part of bran or extract to 10 parts methanol) as previously reported (Beta et al 2005). Samples were extracted at room temperature for 2 h using a wrist shaker at 200 rpm. Methanol extracts were then centrifuged at 3180 x g for 10 min (20 °C) using a bench top centrifuge (Heraeus-Crist, Biofuge A). The free radical scavenging capacity of the supernatant was estimated as follows. A methanol extract (0.1 mL) of wheat bran was added to 3.9 mL DPPH (Sigma-Aldrich, Cat. No. 1898-66-4) solution (6 x 10^{-5} mol/L) and the solution was allowed to stand for 40 min. Absorbance (A) at 515 nm was read against a methanol blank at time 0 and 40
min. The experiments were performed at room temperature and in the dark to prevent oxidation reactions. All tests were carried out in duplicate. The measure of AOA was expressed as % discolouration of the DPPH solution determined as follows:

\[
\text{% decolouration} = \left(1 - \frac{A_{\text{sample} \ t = 30 \text{ min}}}{A_{\text{control} \ t = 0 \text{ min}}} \right) \times 100
\]

In all other AOA related experiments, bran and bran extracts (100 mg) were extracted at room temperature with 1 mL of 50% acetone (ACS grade, Fisher scientific, Cat. No. A18P-4) for 2 h using a RKVSD rotor mixer (ATR Inc., Laurel, MD) at 80 rpm. These extracts were centrifuged at 3180 x g for 10 min (20°C) using the bench top centrifuge. The free radical scavenging capacity of the supernatants was evaluated using DPPH (Sigma-Aldrich, Cat. No. 1898-66-4). DPPH stock solution (6.25 x 10^{-4} mol/L) and working solution (2.08 x 10^{-4} mol/L) were prepared using 50% acetone according to the method described by Liyana-Pathirana et al (2006) and Cheng et al (2006). A bran extract (0.1 mL) was added to 3.9 mL of DPPH working solution and the mixture was vortexed for 10 s. The mixture was incubated at room temperature in the dark for 40 min. Acetone (50%, v/v) was used as a blank to zero the spectrophotometer at 515 nm. Absorbance was read at 515 nm against the blank. A control sample containing no antioxidants was prepared by adding 0.1 mL of 50% acetone to 3.9 mL of working DPPH solution and was incubated with similar conditions as test samples. AOA was expressed as μmol trolox equivalent per g of bran or extract. A standard curve, using trolox (Sigma-Aldrich, Cat. No. 53188-07-1) was prepared for each experiment using 50% acetone with concentration in the range from 0.2-2 mM. Antioxidant
activities were expressed as trolox equivalents in µmol per g of sample. Absorbance difference was calculated as:

\[
\text{Absorbance difference} = \text{Absorbance of control} - \text{Absorbance of sample}
\]

A typical standard curve is shown later in Fig. 4.7.

3.5.2. ABTS free radical scavenging assay

ABTS free radical scavenging was carried out to evaluate antioxidant capacity of bran and bran extracts prepared with different solvents (50% acetone or 50% methanol). Also, this assay was done to evaluate the correlation between DPPH and ABTS based methods. ABTS assay was performed as previously described (van den Berg et al 1999). ABTS (Sigma-Aldrich, Cat. No. 30931-67-0) working solution was prepared by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture was allowed to stand for 12-16 h at room temperature in the dark until it reached a stable oxidative state. On the day of analysis, the ABTS solution was diluted with PBS (phosphate–buffered saline) to an absorbance of 0.700 ±0.01 at 734 nm. For the spectrophotometer assay, 3 mL of the ABTS solution and 20 µl of a standard solution (i.e. extraction solvent) or sample extract were mixed and the absorbance was determined at 734 nm after 20 min incubation at 30 °C. The absorbance value was corrected for the absorbance of an ABTS blank. A blank sample was prepared by adding 20 µl of 50% acetone or 50% methanol to the ABTS solution. A standard curve was prepared using corrected absorbance against trolox concentration in the range 0.2 - 2 mM.
3.5.3. Fractionation of free, esterified and bound phenolics of bran and water extracts of bran for antioxidant activity measurement

Fractionation was done using adaptation of methods described by Krygier et al (1982) and Liyana-Pathirana and Shahidi (2006). Bran or freeze dried bran extract (1 g) was extracted with 10 mL of 50% acetone at room temperature for 2 h in a rotor mixer set to 80 rpm as noted previously. The resulting extract was centrifuged at 795 x g for 10 min. Diethyl ether was added to the supernatant (1:1 v/v) and the mixture was shaken at room temperature for 2 min. This was repeated for six times in total and the upper solvent layer was collected and vacuum dried in a rotary evaporator at 30 °C. The dried residue was re-dissolved in 5 mL of 50% acetone and its AOA was determined using the DPPH free radical scavenging method as described using trolox as the AOA standard. This fraction was considered the free fraction.

The remaining aqueous lower phase of the diethyl ether extraction was treated with 15 mL, 4M NaOH for 4 h at room temperature under N₂ gas using amber-coloured bottles in the dark. The mixture was subsequently acidified to pH ~2 using 6N HCl, and centrifuged at 4926 x g for 10 min using a RC5C Sorvall centrifuge and GSA rotor. To the collected supernatant, diethyl ether (25 ml) was added and the mixture was centrifuged at 10,240 x g (20 °C) for 10 min. This was repeated two times. The upper solvent phase was collected and dried under vacuum in a round bottom flask using a rotary evaporator at 30°C. Dried sample was re-dissolved in 5 ml of 50% acetone and its AOA was determined using the DPPH free radical scavenging method. This fraction was considered as the esterified fraction.
The residue of the initial 50% acetone extract after centrifugation was treated with 15 mL of 4M NaOH for 4 h at room temperature under N₂ gas using amber-coloured bottles in the dark. The mixture was acidified to pH ~2 using 6N HCl and centrifuged at 4926 x g for 10 min. Diethyl ether (20 ml) was added to supernatant and the mixture was centrifuged at 10,240 x g (20 °C) for 10 min. This was repeated two times. The upper solvent phase was collected and dried under vacuum in a round bottom flask using a rotary evaporator at 30°C. Dried sample was re-dissolved in 5 ml of 50% acetone and its AOA was determined using the DPPH free radical scavenging method. This fraction was considered as the bound fraction.

3.6. Determination of phenolic content by the Folin-Ciocalteu method

Phenolic content of bran, extract and residues were determined using a modified version of Folin-Ciocalteu method (Singleton and Rossi 1965, Gao et al 2002). Since phenolic compounds in bran exist in free, esterified and bound form, the phenolic content of these fractions were also determined. A sample (200 mg) was extracted with 4 mL acidified methanol (HCl: methanol: water, 1:80:90 v/v) at room temperature for 2 h using RKVSD rotor mixer (ATR Inc., Laurel, MD) at 80 rpm. The resulting extracts were centrifuged at 795 x g for 10 min using a bench top centrifuge (Heraeus-Crist, Biofuge A). Supernatants (0.2 mL) were oxidized with 1.5 mL Folin reagent, and after 5 min, the mixture was neutralized with 1.5 mL of sodium carbonate (60 g/L). The mixture was then vortexed for 10 s and incubated for 90 min at room temperature in the dark, and its absorbance was measured at 725 nm against a blank (acidified methanol). Ferulic acid (Sigma-Aldrich, Cat. No.537-98-4)
was used as the standard and results were expressed as ferulic acid equivalents per gram of test sample.

3.6.1 Fractionation of free, esterified and bound phenolics of bran and water extracts of bran for phenolic analysis

Fractionation of free, esterified and bound phenolic content of samples was performed essentially as described above for AOA of the fractions, except that samples were extracted with acidified methanol (HCl: methanol: water, 1:80:90 v/v). Resulting fractions were analyzed by the Folin-Ciocalteu method as described above.

3.7. Minolta color determination of bran

Control and heat treated bran samples were evaluated for color using a computerized Minolta color spectrophotometer (Model CM-3500d, Minolta Co., Ltd. Osaka, Japan). Data acquisition and analysis of the colour measurements was performed using the computer software program Spectramagic. The instrument settings for use with supplied optical glass dish were downloaded from the disk provided with the instrument. The 10° standard observer as defined by the CIE (1964) and the D65 (noon daylight) illuminant were used. The colour characteristics measured were CIE L*, a*, b* values. The instrument was calibrated using a reference white tile and black cylinder for 100% and 0% reflectance, respectively. Bran samples were filled up to the top level of the optical glass sample dish and covered with a black coloured cup to ensure background color had no effect on measurements. All analysis was done in duplicate.
3.8. Proximate composition of bran, extracts and corresponding residues.

Samples of control (untreated) and autoclave treated bran along with corresponding subsamples representing freeze-dried water soluble extracts and water-insoluble residues were analyzed in duplicate for content of the following constituents: soluble, insoluble and total dietary fibre (AOAC method 991.43), resistant oligosaccharides (AOAC method 2001.03) inulin or fructans (AOAC method 997.03), β-glucans (AOAC method 995.16), amino acid analysis (Internal method, Medallion Laboratories, Minneapolis, MN), selected mineral composition (wet ashing and ICP-AES, Medallion Laboratories internal method), B-vitamin composition including niacin (B3), thiamine (B1), folic acid (B9), pantothenic acid (B5) and pyridoxine (B6). Vitamin content was determined by Medallion Laboratories.

Resistant oligosaccharides are defined as polysaccharides from DP3-DP10 that are soluble in 80% ethanol and are resistant to digestion by amylase under the conditions of traditional dietary fibre analysis (AOAC 991.43). Accordingly, resistant oligosaccharides are not included in the soluble fibre measurement by AOAC method 991.43. They were ultimately quantified by size-exclusion HPLC.

3.9.1. Protein determination of bran and extracts

A Micro Kjeldahl method (AACC 46-13) was used to determine the protein content (N x 5.7) of the fine bran, extracts and bran residues using 100 mg of material.

3.8.2. Moisture content determination

Moisture content of fine bran, extracts and residues were determined using AACC air-oven method (AACC 44 -15 A).
3.8.3. Total carbohydrate analysis

The monosaccharide composition of wheat bran and wheat bran extracts was determined (in duplicate) by high performance anion exchange chromatography (HPAEC). Primary hydrolysis of the samples was performed with 12M H₂SO₄ at 35°C for 1 h. Hydrolysates were diluted to 1M H₂SO₄ with distilled water. Fructose obtained from Sigma (St. Louis, MO) was added as an internal standard and a secondary hydrolysis was performed at 100 °C for 2 h. The hydrolysates were diluted with water so that the sample sulphate load was 2 micromoles or less with a 20 μl injection. Standard monosaccharides (fructose, arabinose, galactose, glucose, xylose, mannose) were obtained from Sigma (St. Louis, MO). To correct for sugar degradation during secondary hydrolysis, system calibration was based upon a standard mixture of the monosaccharide treated in parallel with each batch of samples.

To determine cellulose content, the samples were also hydrolyzed with 1M H₂SO₄ at 100°C for 2 h and the difference in glucose between the two hydrolysates was calculated as cellulose. Samples were filtered through a 0.45 um GHP acrodisc syringe filter (PALL corporation, Mississauga, ON) and analyzed by HPAEC using a Waters 625 pump LC system, Waters 717 plus autosampler (Waters Corporation, Milford, MA), a Dionex Carbopac PA1 column (4 x 250 mm), a PA1 guard column (4 x 50 mm) (Dionex Canada Ltd., Etobicoke, ON) and a Coulochem III pulsed amperometric electrochemical detector equipped with a 5040 analytical cell containing a gold target electrode (ESA, Inc., Chelmsford, MA). The column was kept at 30°C and the hydrolyzed samples were eluted at 1.0 mL/min with nanopure water for 11 min followed by a 2 min ramp to 200 mM NaOH with 170mM sodium acetate which was maintained for 5 min to wash the column. Over a 2
min period the column was then brought back to the original starting conditions and allowed to equilibrate for 9.5 min prior to the next sample being injected. Total run time was 29.5 min. Concentrated base (300 mM NaOH) was added to the post-column effluent at 0.5 mL/min with a Model 584 Pump (ESA, Inc., Chelmsford, MA). The coulochem III detector pulse parameters were E1 = +100 mV, E2 = -1500 mV, E3 = +600 mV, E4 = -100 mV; T1 = 500 ms, acquisition delay (AD) = 300 ms, T2 = 10 ms, T3 = 1 ms, T4 = 40 ms, current range = 2 uc; recorder out at +1 V; and a baseline offset of 0%. Total neutral carbohydrates were calculated based on this monosaccharide results.

3.8.4. Non-starch polysaccharide content of bran and extracts

Two major components of NSP of wheat bran were specifically determined, i.e. β-glucans and arabinoxylans. β-glucan content of treated bran, extracts and residues was measured using AACC Approved Method 32-22, and results were adjusted to 14% moisture basis. Total arabinoxylan (pentosans) was determined using the colorimetric method of Bell (1985) with pre-hydrolysis using 0.5 M sulphuric acid (Wang et al 2006).

3.8.5 Starch assay

Starch assay was done using Megazyme starch kit. This method followed the standard procedure of AACC method 76.13 or AOAC method 996.11.

3.8.6. Determination of phytic acid

Phytic acid content was determined as described by Latta et al (1980). Fine bran, extracts and residues (0.25 g) were treated in 25 mL flasks with 5 mL 2.4% HCl. The
contents of the flasks were mixed for 1 h using a magnetic stirrer. The mixtures were then centrifuged at 10,240 x g (20°C) for 10 min using a GSA rotor and RC5C Sorvall centrifuge (DuPont Co. Newtown, CT) and a clear supernatant was obtained.

A glass anion exchange column was prepared using 0.5 g 200-400 mesh glass wool and AG1-X8 chloride anion exchange resin (Biorad). The column was prepared with 15 mL 5% HCl and then rinsed with 20 mL deionised water. Supernatant (1 mL) of each sample was diluted in a 25 mL volumetric flask with deionised water. The diluted sample (10 mL) was added to the column using a pipette. Once the sample had passed through, 15 mL of 0.1 M NaCl was added. Once the NaCl had passed through, the eluent was discarded and 15 mL 0.7 M NaCl was added to the column and the new eluent was collected in a clean flask. This was repeated for each diluted sample. The collected samples were diluted to 25 mL with distilled water. Wade reagent (1 mL, 0.9 g anhydrous FeCl₃ and 1.5 g sulphosalicylic in 500 mL in water) was added to each of the 3 mL of collected diluted samples which were then vortexed. The absorbance of the resulting mixture was measured at 500 nm using water as a blank. Phytic acid was used as the standard. All tests were carried out in duplicate.

3.8.7. Determination of enzyme activity of bran

The ability of various bran treatments to deactivate enzyme activity in the bran was tested on the following samples: control, autoclave dry, autoclave wet, oven-air dry, and oven-air wet. Polyphenol oxidase (PPO) activity was determined according to the method of Hatcher and Kruger (1993) using catechol as the substrate. Peroxidase (POD) activity was determined using 1-Step ABTS (2, 2’-azine-di [3-ethylbenzthiazoline sulfonate]) (Pierce) as the substrate and measured at 405 nm using a kinetic microplate reader (Thermomax,
Molecular Devices Corp., Sunnyvale, CA) at a constant temperature of 20 °C (Hatcher and Barker 2005).

3.8.8. Statistical analysis

Selected results were analyzed by analysis of variance (ANOVA). The ANOVA was performed with the one way ANOVA Tukey test of the “Graphpad Instat” software package (Graph pad software, California, USA). All the treatment effects were compared to control bran and extracts. Duplicate analyses were used. Comparison of means for differences was done at the 5% significance level using the Tukey multiple comparison test.
4. RESULTS AND DISCUSSION

4.1. Particle size effect

Effect of three different bran particle sizes (coarse, fine and ultrafine) on dough rheological properties antioxidant activity and total phenolic content were determined.

4.1.1. Particle size effects on dough rheological properties

Mixograph (10 min duration mixing curves) of the three bran particle sizes did not show any significant differences (data not shown). All three particles sizes produced a strong-looking mixogram, with dough break down times between 3.5 – 4.5 min.

Regardless of the particle size, autoclaved wet bran produced much stronger mixing curves compared to the autoclaved dry bran. Compared to control samples, autoclaved bran (dry and wet) can produce satisfactory doughs. Interestingly, compared to a whole wheat dough with untreated bran, autoclaved dry bran produced a weakening effect in the mixograph (reduced mixing time and increased breakdown after the peak), whereas autoclaved wet bran produced a noticeably stronger dough with increased mixing time (Fig. 4.1). The latter result appears to be similar to that obtained by Nelles et al (1998) with hydrated bran added to a base flour (12% flour replacement) and mixed in a farinograph; the dough had longer development times and greater stabilities. However in that study, bran was soaked with excess water and was then wrung out to expel water before being tested. In the present study, wheat bran was hydrated with a much lower amount of water (20 g bran and 10 mL water). Wetted control bran was not evaluated in this study.
When bran extracts were evaluated in the same way, a very different result was obtained (Fig. 4.2). In this case, adding the water soluble extract from untreated bran to base flour resulted in no dough formation whatsoever. In contrast, corresponding extracts from autoclave dry and wet bran produced normal looking mixograms very similar in appearance to that obtained by adding autoclave dry bran to a base flour (Fig. 4.1, top). The absence of dough formation when the extract of untreated bran was added to the base flour is likely due to protease or more likely NSP degrading enzymes (e.g. xylanase) present in the aqueous extract, as extracts from autoclaved bran appeared to lack any enzyme activity (Figs. 4.29, 4.30) at least in regard to polyphenol oxidase and peroxidase. McCleary (1986) has demonstrated that adding small amounts of xylanase enzyme to wheat flour dough can completely and almost immediately destroy normal dough mixing properties by hydrolysis of arabinoxylans which have significant water binding properties disproportionate to their relatively low concentration in wheat endosperm and flour (Bushuk 1966). Wheat bran contains xylanase enzymes which are extractable in water (Van Craeyveld et al 2010) and can be effectively deactivated by normal autoclave conditions (Van Craeyveld et al 2010).

The dough mixing properties of flour could be additionally changed with addition of autoclaved bran extracts depending on the temperature of extraction of the water solubles. As extraction temperature was increased, dough strength was also increased (Fig. 4.3) judging from the increase in mixing time to peak and bandwidth of the dough mixing curves. The nature of this effect was not explored further as the composition of the bran extracts prepared at different temperatures was not determined. It could be speculated that increasing the temperature of extraction of bran might increase arabinoxylan content of the extract, thereby making the doughs appear stronger at constant absorption that was used in the mixing
procedure. However, increasing the amount of autoclaved bran extract in the dough mix did not produce a clear trend of increasing dough strength (Fig. 4.4), as it would appear that adding extract to a dough at the 5% level produced a noticeably weaker dough compared to adding only 2% of extract to a base flour.

4.1.2. Effect of bran particle size on antioxidant activity and phenolic content of extracts

The effect of bran particle size on antioxidant activities (AOA) of methanolic extracts of bran, water-soluble extracts and residues depended on the sample (Table 4.1). For bran material, the highest AOA was obtained for the finest bran samples regardless of treatment. As is discussed below, bran exposure to oxygen appears to play a role in AOA outcomes of extracts; the lower the oxygen exposure, the higher the AOA. It seems plausible that as bran becomes finer, it becomes more prone to oxidation at a given storage temperature. Accordingly, the finest bran might be expected to have the lowest AOA. However, the opposite was observed suggesting increased surface area of bran with decreasing particle size promoted the extraction of compounds with AOA. On the other hand, for freeze dried water soluble extracts, that trend was reversed, i.e. the finer the starting bran, the lower the AOA of freeze dried water soluble extracts. However, the total phenolic content of extracts (Table 4.2) did not decrease with decreasing bran particle size. For residue material the effect of bran particle size was generally smaller than that found for bran and extracts, and also inconsistent (Table 4.1).
Figure 4.1. Effect of adding untreated and autoclaved bran (15% added to base flour) in the mixograph.
Figure 4.2. Effect of adding freeze-dried water soluble extracts of control and autoclaved bran (15% added to base flour) in the mixograph.
Figure 4.3. Effect of adding autoclaved bran extracts (10% added to base flour) extracted from bran at different temperatures on mixograph properties.
Figure 4.4. Effects of adding water soluble extracts of autoclaved bran (extraction temperature, 20 °C) on mixograph properties at indicated addition levels to a base flour.
The effect of bran particle size on phenolic content of bran, extracts and residues was essentially insignificant (Table 4.2). While AOA tended to decrease for extract material with decreasing bran particle size, the main differences were between coarse and fine bran, except for autoclaved moist bran where AOA of ultrafine bran extracts were significantly lower than that for fine bran. In general however, fewer AOA differences were seen between the fine and ultrafine bran. As fine bran was of a particle size consistent with wheat milling industry standards for wheat bran (Ashok Sarkar, personal communication) and this bran was easy to prepare and handle, all subsequent experiments were carried out using fine bran particle size. Moore et al (2009) studied particle size effect on AOA of whole wheat pizza crust. Their results showed no effect of particle size on AOA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample</th>
<th>Coarse</th>
<th>Fine</th>
<th>Ultrafine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bran</td>
<td>16.55 ± 1.91</td>
<td>22.55 ± 0.21</td>
<td>23.60 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>Extracts</td>
<td>26.45 ± 3.46</td>
<td>20.75 ± 1.34</td>
<td>20.30 ± 3.68</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>11.65 ± 0.78</td>
<td>10.45 ± 0.07</td>
<td>9.25 ± 0.78</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>Bran</td>
<td>23.15 ± 0.78</td>
<td>24.80 ± 0.28</td>
<td>26.80 ± 0.85</td>
</tr>
<tr>
<td>(Moist)</td>
<td>Extracts</td>
<td>56.10 ± 1.70</td>
<td>50.40 ± 1.13</td>
<td>42.50 ± 1.70</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>18.35 ± 0.07</td>
<td>20.10 ± 0.57</td>
<td>15.90 ± 0.57</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>Bran</td>
<td>21.05 ± 3.46</td>
<td>22.90 ± 0.14</td>
<td>24.30 ± 3.39</td>
</tr>
<tr>
<td>(Dry)</td>
<td>Extracts</td>
<td>62.85 ± 1.20</td>
<td>56.30 ± 1.41</td>
<td>58.95 ± 2.05</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>12.30 ± 3.39</td>
<td>16.95 ± 1.34</td>
<td>14.05 ± 0.78</td>
</tr>
</tbody>
</table>

* Antioxidant activity was determined by the DPPH % decolourization assay using methanolic extracts. Higher values denote higher activity. Values are expressed as mean ± standard deviation.
Table 4.2. Effect of bran particle size and autoclaving on total phenolic content (ferulic acid equivalents mg/g sample) of bran, water-soluble extracts and residues.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample</th>
<th>Coarse</th>
<th>Fine</th>
<th>Ultrafine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Bran</td>
<td>5.14 ± 0.18</td>
<td>5.48 ± 0.06</td>
<td>5.09 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>Extracts</td>
<td>12.03 ± 0.34</td>
<td>11.49 ± 0.11</td>
<td>11.74 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>2.29 ± 0.04</td>
<td>2.17 ± 0.15</td>
<td>0.98 ± 0.13</td>
</tr>
<tr>
<td>Autoclaved (Moist)</td>
<td>Bran</td>
<td>4.80 ± 0.30</td>
<td>5.19 ± 0.59</td>
<td>5.28 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>Extracts</td>
<td>13.99 ± 0.30</td>
<td>13.94 ± 0.01</td>
<td>14.10 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>2.17 ± 0.17</td>
<td>2.82 ± 0.57</td>
<td>2.85 ± 0.02</td>
</tr>
<tr>
<td>Autoclaved (Dry)</td>
<td>Bran</td>
<td>4.68 ± 0.12</td>
<td>4.94 ± 0.27</td>
<td>5.16 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Extracts</td>
<td>15.87 ± 0.46</td>
<td>16.15 ± 0.30</td>
<td>16.50 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>Residue</td>
<td>2.30 ± 0.03</td>
<td>1.91 ± 0.06</td>
<td>1.95 ± 0.10</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation.

4.2. Heat treatment effects

4.2.1. Effect of different bran treatments on antioxidant activity and total phenolic content of bran, extracts and residues

Untreated bran and extracts of untreated bran possessed comparable levels of AOA, about double that of residue material (Table 4.3). For bran material, autoclaving resulted in a negligible effect on the AOA of methanolic extracts of bran, while oven heating clearly reduced the AOA levels of bran even lower than that of untreated bran. No similar result was obtained for water soluble extracts of bran. What is one of the most remarkable and compelling results of this study is the dramatic effect on AOA caused by autoclaving, and oven-heating in the presence of N₂. These treatments resulted in a 240-280% increase in AOA levels of bran extracts in this experiment. Other air-oven heating treatments (both wet
and dry bran) and N₂ atmosphere oven treatment of wet bran produced extracts with the lowest AOA levels. These results suggest that heat treating bran in the absence of O₂ is a key processing factor that produces the substantial enhancement of AOA in water extracts of bran. While this outcome seems intuitive for the N₂ oven treatment of dry bran, the corresponding results for autoclaving and N₂ oven treatment of wet bran are less obvious. For autoclaving, it is plausible that the moderate overpressure of 22 PSI and steam environment in which bran is exposed are the key factors that would effectively and rapidly eliminate O₂. Presumably wetting the bran quickly initiates oxidation reactions (that denature constituents with AOA) that are very rapidly accelerated by exposure to heat in the air-oven notwithstanding the N₂ rich atmosphere. The significantly lower AOA of autoclaved wet bran extracts compared to dry bran counterparts would seem to support this concept. It would appear then that exposure of wheat bran to water should be avoided in order to maximize the AOA of bran extracts and in turn the potential efficacy of autoclaved bran as a functional food.

Table 4.3. Effects of different bran heat treatments on the antioxidant activity of bran, water soluble extracts and residues.

<table>
<thead>
<tr>
<th>Heat Treatment</th>
<th>Bran</th>
<th>Extract</th>
<th>Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.55a ± 0.21</td>
<td>20.75a ± 1.34</td>
<td>10.45a ± 0.00</td>
</tr>
<tr>
<td>Autoclaved (dry)</td>
<td>22.90a ± 0.14</td>
<td>56.30b ± 1.41</td>
<td>16.95b ± 1.34</td>
</tr>
<tr>
<td>Autoclaved (wet)</td>
<td>24.80a ± 0.28</td>
<td>50.40b ± 1.13</td>
<td>16.95b ± 1.34</td>
</tr>
<tr>
<td>Oven heated (air) dry</td>
<td>16.10b ± 2.67</td>
<td>7.85d ± 0.00</td>
<td>11.95a ± 0.10</td>
</tr>
<tr>
<td>Oven heated (air) wet</td>
<td>14.51b ± 2.36</td>
<td>11.96e ± 0.71</td>
<td>10.45a ± 0.57</td>
</tr>
<tr>
<td>Oven heated (N₂) dry</td>
<td>15.44b ± 0.19</td>
<td>48.22c ± 0.00</td>
<td>8.31a ± 0.00</td>
</tr>
<tr>
<td>Oven heated (N₂) wet</td>
<td>13.05b ± 0.62</td>
<td>7.74d ± 0.00</td>
<td>11.38a ± 0.00</td>
</tr>
</tbody>
</table>

Antioxidant activity was determined by the DPPH % decolourization assay using methanolic extracts. Higher values denote higher activity. Values expressed as mean ± Standard deviation. Mean in same column followed by different letters are significantly different (p<0.05)
Similar results were obtained in a separate experiment (Fig. 4.5) except that in this case, only water soluble extracts of autoclaved dry bran showed substantially enhanced AOA expressed relative to the AOA of a standard preparation of the synthetic antioxidant trolox. Unlike the earlier experiment (Table 4.3), pre-treating bran in an air oven with N\textsubscript{2} did not produce an AOA enhancing effect. Reasons for the difference in outcomes of these two experiments may be related to different specificities of the different organic solvents used to extract the antioxidants; 100\% methanol and 50\% aqueous acetone in the former (Table 4.3) and latter (Fig. 4.5) experiments, respectively. Figure 4.6 shows a typical trolox standard curve for AOA determination; response was highly linear across the absorbance range used.

Figure 4.4. Effects of different bran treatments on the antioxidant activities (trolox equivalents umol/g sample) of bran and freeze dried water soluble extracts. Averages of bran and extracts marked by the same letter are not significantly different (p<0.05). N12 and N25 refer to N\textsubscript{2} gas flow rates of 12 and 25 L/min, respectively.
Analysis of total phenolic content of bran, extracts and residues, likewise indicated that among all the heat treatments, autoclaving of dry bran and to a somewhat lesser extent for autoclaved wet bran, yielded water soluble extracts with distinctly high levels of total phenolics (Table 4.4). All bran samples and all residue samples obtained from all of the heat treatments, had similar levels of total phenolics as their respective controls. Comparing AOA and total phenolic content results in Tables 4.3 and 4.4, respectively indicate that antioxidant activity enhancement of water soluble extracts of autoclaved bran compared to control samples is not well correlated with phenolic content results, although the latter also increase significantly. Evidently the increase in AOA of the autoclaved bran, and especially that of autoclaved dry bran, is not due to an enhancement in extractability or solubility of phenolic compounds only. Therefore, there are other compounds that are responsible for the increased antioxidant activity in these autoclaved samples.
Table 4.4. The effects of different bran heat treatments on the total phenolic content (ferulic acid equivalents mg/g sample) of bran, water soluble extracts and residues.

<table>
<thead>
<tr>
<th>Heat Treatment</th>
<th>Bran</th>
<th>Extract</th>
<th>Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.46a ± 0.07</td>
<td>11.07c ± 0.56</td>
<td>2.39b ± 0.14</td>
</tr>
<tr>
<td>Autoclaved (dry)</td>
<td>4.94a ± 0.14</td>
<td>16.15d ± 0.30</td>
<td>1.91b ± 0.06</td>
</tr>
<tr>
<td>Autoclaved (wet)</td>
<td>5.19a ± 0.59</td>
<td>13.94e ± 0.01</td>
<td>2.82 b± 0.12</td>
</tr>
<tr>
<td>Oven heated (air) dry</td>
<td>5.48a ± 0.06</td>
<td>11.49c ± 0.11</td>
<td>2.17 b± 0.15</td>
</tr>
<tr>
<td>Oven heated (air) wet</td>
<td>5.78a ± 0.19</td>
<td>12.28d ± 0.32</td>
<td>2.02b ± 0.09</td>
</tr>
<tr>
<td>Oven heated (N₂) dry</td>
<td>5.53 a± 0.23</td>
<td>11.72c ± 0.08</td>
<td>2.14b ± 0.02</td>
</tr>
<tr>
<td>Oven heated (N₂) wet</td>
<td>5.93 a± 0.19</td>
<td>10.54c ± 0.14</td>
<td>2.42 b± 0.04</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation. Means in same column followed by different letters are significantly different (p<0.05).

4.2.2. Effect of repetitive autoclaving of bran on antioxidant activity of bran extracts

Repeating the basic autoclave conditions in succession resulted in progressive increase in AOA of water soluble extracts (Fig. 4.7). However, the major effect occurred after the initial autoclave cycle at which time AOA increased by ~ 500% compared to untreated bran. With each autoclave cycle bran colour also progressively darkened, and the aroma of the bran changed from a very pleasant nutty and cooked grain aroma after one autoclave cycle, to a pungent aroma consistent with burnt food.

4.2.3. Effect of increasing sterilization time (without drying cycle) on antioxidant activity of bran extracts

AOA of wheat bran extracts was evaluated for different sterilization times without a drying cycle. It can be seen that the predominant effect of autoclaving on AOA of bran extracts occurred after only 5 min of treatment at 121 oC and 22 psig (Fig. 4.8), which represented one-third of the standard 15 min sterilization time in the autoclave (Fig. 3.1).
Figure 4.6. Effect of repetitive autoclaving of bran on antioxidant activity of bran extracts.

Figure 4.7. Effect of autoclaving sterilization time from 5 to 60 min without drying cycle on antioxidant activity of bran extracts.
Additional sterilization time had little effect on increasing AOA of bran extracts. However, as was noted previously with increasing number of autoclave cycles, increasing sterilization time caused the bran to darken in colour and take on a strong burnt product aroma. Comparing results in Figs. 4.7 and 4.8 suggest that repeating autoclave cycles can produce higher levels of AOA compared to increasing the autoclave time. The main difference between these two treatment procedures is that repeating autoclave cycles is accompanied by repeating cycles of decompression as the steam content of the autoclave is evacuated under reduced pressure (Fig. 3.1). It has been suggested that rapid decompression of a food material may cause cellular damage due to rapid expansion of gas that was dissolved during pressurisation (Norton and Sun 2008). It seems plausible that this factor is at least partially responsible for enhancement of extractability of soluble bran antioxidants in this thesis research.

4.2.4. Effect of increasing sterilization time (with drying cycle) on antioxidant activity of bran extracts.

The effect of increasing sterilization time with drying cycle on antioxidant activity of bran extracts was essentially identical to results obtained without drying (Fig. 4.9). In this experiment, total sterilization time was increased from 60 min (Fig. 4.8) to 90 min. As before, the predominant effect of autoclaving on AOA of bran extracts occurred after only 5 min of treatment. As well, results for Platte bran followed the same trend as for CWRS wheat bran, although AOA levels for Platte bran were slightly lower.
The bran colour was very dark brown after 90 min of sterilization time in the autoclave for the red-coloured CWRS wheat bran. Even for the white-branned Platte bran, colour of the product was only marginally lighter. In fact it seemed like a fair observation that bran treated in the autoclave for more than 20-30 min would not be acceptable for a food application as its colour and aroma would most likely be deemed unacceptable. The results suggest that autoclaving bran at 121 °C for 10-15 min sterilization with drying cycle is close to ideal to obtain desired bran colour and aroma together with increased solubilisation of antioxidant compounds.
4.2.5. Effect of sterilization temperature on antioxidant activity of bran extracts

The autoclave used in this study was capable of two different temperatures only, 121 °C and 132 °C. As in previous experiments, compared to untreated bran, autoclaving bran at the standard temperature of 121 °C resulted in very considerable increase in AOA of both CWRS and Platte wheat bran extracts (Fig. 4.10), with significantly higher AOA for the Platte bran. Increasing the sterilization temperature to 132 °C resulted in a further increase in AOA of extracts only for CWRS bran. It is unclear why the different brans responded differently in this way.

![Figure 4.9](image_url)

Figure 4.9. Effect of autoclave temperature on antioxidant activity of CWRS and Platte wheat bran extracts. Averages marked by the same letter are not significantly different (p<0.05).
4.3. Optimization of extraction conditions for antioxidant activity determination

Previous studies (Oufnac et al. 2007; Liyana-Pathirana and Shahidi 2005; Zhou and Yu 2004) indicated variable effects of extraction solvent and related conditions on AOA of wheat and bran. In the present research, optimization of extraction conditions for solvent type, temperature, and time was carried out using DPPH and ABTS free radical scavenging methods.

4.3.1. Effect of extraction solvent on antioxidant activity using ABTS radical scavenging

Two different solvents (50% methanol and 50% acetone) were used to extract antioxidants from bran extracts and bran residues. Control bran exhibited no significant difference of AOA in response to different solvents used (Tables 4.5 and 4.6). Also, there was little difference due to solvent in extracts and residues from autoclaved dry and wet bran. For the autoclaved dry treatment, it appeared that 50% acetone yielded higher AOA of both extracts and residues (Table 4.6) than corresponding results for methanol (Table 4.5). Previous studies (Liayana-Pathirana and Shahidi 2005; Zhou and Yu 2004) reported that 50% acetone was the better extractant to perform free radical scavenging tests.

4.3.2. Effect of extraction solvent on antioxidant activity using DPPH radical scavenging

In this experiment, two different brans (CWRS and Platte) were evaluated in response to AOA determination using three different solvents (methanol-acetone-water, 50% acetone, and 70% methanol). For control and autoclave treated bran, there was a small significant difference in AOA due to solvent (Fig. 4.11).
Table 4.5. Effect of 50% methanol extraction on antioxidant activity (ABTS radical scavenging) of autoclaved bran water soluble extracts and residues (Trolox equivalent mmol/g sample).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bran Extracts</th>
<th>Bran Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.54a ± 0.00</td>
<td>0.42a ± 0.40</td>
</tr>
<tr>
<td>Autoclave Dry</td>
<td>5.87b ± 0.48</td>
<td>2.07b ± 0.49</td>
</tr>
<tr>
<td>Autoclave wet</td>
<td>3.58c ± 0.85</td>
<td>2.98b ± 0.44</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation. Mean values in the same column followed by different letters are significantly different (p<0.05).

Table 4.6. Effect of 50% acetone extraction on antioxidant activity (ABTS radical scavenging) of autoclaved bran water soluble extracts and residues (Trolox equivalent mmol/g sample).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bran Extracts</th>
<th>Bran Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.46a ± 0.00</td>
<td>1.99a ± 0.59</td>
</tr>
<tr>
<td>Autoclave Dry</td>
<td>6.89b ± 0.01</td>
<td>2.68b ± 0.20</td>
</tr>
<tr>
<td>Autoclave wet</td>
<td>3.19c ± 0.78</td>
<td>3.07c ± 0.55</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation. Mean values in the same column followed by different letters are significantly different (p<0.05).

For both control and autoclaved brans, 70% ethanol as a solvent extracted small but significantly higher AOA compared to 50% acetone (Figs. 4.11, 4.12). For extracts from untreated bran, there was likewise little difference in AOA due to extraction solvent, although for Platte bran, methanol-acetone-water yielded significantly higher AOA (Fig. 4.12). For extracts from autoclaved bran, there was again little difference among solvents in AOA outcomes. For freeze dried water soluble extracts from Platte bran, 50% acetone gave a
slightly higher level of AOA compared to the other two solvents (Fig. 4.11). Once again, autoclaved bran produced water soluble extracts with remarkably higher levels of AOA compared to corresponding extracts from untreated bran. After considering these results, 50% acetone was selected as the solvent for subsequent experiments. Zhou and Yu (2004) evaluated similar solvents (50% acetone, 100% ethanol, 70% ethanol, and 70% methanol) and concluded that based on results for total phenolic content and AOA, 50% acetone was the preferred solvent to prepare antioxidants extracts from wheat bran for routine analytical work.

Figure 4.10. Effect of different extraction solvent on antioxidant activity (DPPH radical scavenging) of CWRS bran and extracts. Mean values across sample types with the same letters are not significantly different (p<0.05).
4.3.3. Effect of extraction temperature on antioxidant activity and phenolic content of bran extracts

Compared to the major effect of enhancement of AOA of water soluble bran extracts due to autoclave treatment, the effect of different extraction temperatures (4, 20 and 50 °C) on AOA (assessed by DPPH decolourization assay, Fig. 4.13) and total phenolic content (Fig. 4.14) was comparatively small. On this basis, ambient temperature or 20 °C appears to be a very convenient condition for preparing water-soluble extracts of bran in routine work. Oufnac et al (2007) reported progressive increase of total phenolic content and AOA of
Figure 4.12. Effect of extraction temperature on antioxidant activity (DPPH radical decolourization) of bran extracts. Means of different treatments marked by the same letter are not significantly different (p<0.05).

Figure 4.13. Effect of extraction temperature on total phenolic content (ferulic acid equivalents) in bran extracts. Averages of different treatments marked by the same letter are not significantly different (p<0.05).
wheat bran extracts with methanol as temperature of extraction in sealed vessels was increased above 60 °C (for AOA) and 80 °C (for total phenolic content) to 100 °C and 120 °C in a microwave oven. Oufnac et al (2007) reported no difference in AOA of bran extracted with methanol between 60 °C at ambient pressure and 60 °C in sealed vessels. Evidently extracting wheat bran at high temperatures (>60 °C) in sealed containers can significantly increase extraction of phenolic compounds and AOA. In the same study, there appeared to be a close correspondence or correlation between total phenolic content and AOA using different solvents and extraction temperatures. For material analyzed in this thesis experiment, no close correspondence between AOA and total phenolic content was obtained (compare Figs. 4.13 and 4.14). It can be seen that the autoclave effect was much greater to enhance extraction of antioxidant compounds with free radical scavenging properties (Fig. 4.13) compared to phenolic compounds evaluated by the Folin-Ciocalteu method (Fig. 4.14). As observed previously, the Folin method may not accurately estimate phenolic compounds and/or compounds with AOA extractable in water may not all be phenolic in nature.

4.3.4. Effect of extraction time on antioxidant activity of wheat bran extracts

Effect of extraction time on AOA of bran extracts was evaluated from 1 to 36 h at room temperature using both CWRS and Platte wheat brans. Results (Fig. 4.15) show that extraction time produced some interesting contrasts between control and autoclave treated bran. By extending the extraction period to 36 h, it can be seen that the initial large difference in AOA between untreated and autoclave treated bran gradually diminishes. The increase in
AOA of autoclaved bran appears at 18 h, whereas for control bran, another 8 h of extraction was required for increases in AOA to become evident. This earlier increase in AOA for autoclaved bran most likely arises from its more soluble nature compared to control bran. However, by 36 h, most of the difference in AOA between the two types of bran was largely eliminated. For control bran, AOA of water soluble extracts continues to increase. At this point in time (even at 16 h) the water suspensions of the untreated bran showed significant evidence of fermentation as suspensions had a strong aroma of organic acids, particularly acetic, and were visibly bubbling. No doubt, enzyme activity was considerable for the untreated bran extracts which likely also contributed to increased solubilisation of AOA compounds reflected in the increasing AOA. In contrast, the autoclaved bran suspensions showed no evidence of natural fermentation, their aroma was pleasant with no hint of organic acid production and as is shown later, enzyme activity was likely nil.
4.4. Colour of bran undergoing thermal treatments

Heat treatment effects on bran color were evaluated using a computerized colour spectrophotometer based on computed L*, a* and b* values. Increasing and decreasing L* values in the range 0-100 correspond to increasing and decreasing brightness or reflectance, respectively in the range of black to white. Increasing yellow and blue colour is represented by positive and negative b* values, respectively, whereas increasing red and green colouration is represented by positive and negative a* values, respectively. As is detailed below, autoclaving of bran clearly resulted in lowering of L* values and increasing of both a* and b* values which was consistent with a darkening and more brown coloured bran product.
Increasing autoclave time on CWRS wheat bran colour resulted in a progressive darkening of the material as reflected by decreasing L* values and increasing values for a* and b*. The major effect was observed after only 5 min of sterilization plus 10 min drying time (Fig. 4.16), however the bran continued to darken and become more brown in colour with increasing degree of autoclaving. Likewise, increasing the number of basic autoclave cycles (Fig. 4.17) or increasing autoclave temperature (Fig. 4.18) had similar effects on bran colour. Clearly increasing the duration or intensity of autoclaving resulted in a darker coloured product. A Maillard browning effect is presumed to be responsible for this result. It was interesting that a very close correspondence existed between bran colour and levels of AOA in water soluble extracts to increasing bran autoclave time (compare Figs. 4.9 and 4.16). Interestingly, heating of bran in an air-oven at the same temperature and for the same 15 min sterilization time frame as for autoclaving (Fig. 4.19) had no effect on bran
Figures 4.15. Effect of autocar and after treatment on the color value (L* Value, a* Value, b* Value) of Control and AD samples. The results are shown as means ± standard deviations.
Figure 4.16. Effect of repetitive autoclave cycles on bran color.
Figure 4.17. Effect of different autoclave temperatures on bran color.
Figure 4.18. Effect of heat treatment on bran color. “Auto” denotes autoclaved bran. Oven N12 and N25 denote heating of bran in the air oven flushed with N₂ gas at 12 and 25 L per min. Averages of heat treatments marked by the same letter are not significantly different (p<0.05).
reflectance (L* values) compared to untreated bran, and had a small but significant effect on reducing the yellow (b*) and red (a*) colouration of the CWRS wheat bran which was opposite to the effect of autoclaving on bran colour. Clearly, autoclaving of bran with its pressure component and oxygen-free environment represents a form of hydrothermal treatment very different from oven heating or roasting.

As noted above, extending the degree of autoclaving beyond 15-20 min of sterilization time results in bran appearance and aroma which, for red-coloured bran such as CWRS wheat, would likely be unacceptable to the food industry for sensory reasons. In fact, white coloured bran such as Platte produced bran after one autoclave cycle with a very attractive golden colour as assessed subjectively. For this reason, soft white or hard white wheat bran may be the ideal types of bran for commercial application of autoclaving to enhance bran functional properties in food.

4.5. Free, esterified and bound phenolic fractions of bran

4.5.1. Effect of autoclave treatment on content of free, esterified and bound phenolic fractions of bran and extracts, and their antioxidant activities

Phenolic compounds in wheat bran can be considered to be in three different forms or fractions: soluble free phenolics, soluble phenolics esterified or otherwise conjugated to carbohydrates and insoluble phenolics that are bound to cell wall polysaccharides or lignin. Phenolic acids may form both ester and ether bonds due to their bifunctional nature through reactions involving their carboxyl and hydroxyl groups, respectively. This allows phenolics acids to form cross-links with cell wall macromolecules. Ferulic acid is the major phenolic
acid in wheat bran (Sosulski et al 1982, Hatcher and Kruger 1997), which is predominantly in bound form.

Previous results reported above indicated that autoclaving of wheat bran produced ~50% increase in total phenolic content (TPC) of water soluble extracts compared to corresponding extracts of untreated bran (Tables 4.2, 4.4). Analysis of free, esterified, and bound phenolic fractions (Fig. 4.19) indicate that the greater majority of the increase in TPC of water-soluble extracts was likely due to increased extractability of esterified phenolics, for which there was ~ four-fold increase compared to soluble esterified phenolic content of water-soluble extracts of control untreated bran. Interestingly, there appeared to be no correspondence between the phenolic fractionation results and corresponding AOA results for the same fractions (Fig. 4.20). A possible reason for this could be the different solvents

![Figure 4.19](#)

**Figure 4.19.** Effects of bran autoclaving on free, esterified and bound phenolics of water-soluble bran extracts. Averages of treatments marked by the same letter are not significantly different (p<0.05).
used at the start of the fractionation for AOA (50% acetone) and phenolic content (acidified methanol) determinations. It should also be emphasized that for the material under analysis, i.e. freeze dried water soluble extracts of bran, by definition there is no bound phenolic content, as there would be for bran, which presumes that the material is largely insoluble in nature. Accordingly, the results in Figs. 4.19 and 4.20 may be artifactual as far as the separate fractions are concerned. In contrast, there can be no doubt from earlier results that autoclaving has a remarkable effect to enhance the AOA of water-soluble extracts of wheat bran (Tables 4.1-4.4, Figs. 4.5, 4.9, 4.10-4.13), and to a lesser extent, total phenolic content (Fig. 4.14) which is basically what Figs. 4.19 and 4.20 reflect when the totality of results are considered.

Figure 4.20. Effects of bran autoclaving on antioxidant activity of free, esterified and bound phenolic fractions of water-soluble bran extracts. Averages of treatments marked by the same letter are not significantly different (p<0.05).
Corresponding results for wheat bran were different (Figs. 4.21, 4.22) where little difference was found between control and autoclaved bran in either phenolic content (Fig. 4.21) or AOA (Fig. 4.22) of free, esterified and bound phenolic fractions. The only notable exception was a fairly substantial increase of over 40% in AOA of free phenolics of autoclaved bran compared to untreated counterpart, from \(~4.4\) to \(~6.3\) µmol trolox equivalents/g, respectively. However, considering all three fractions, this increase in AOA due to autoclaving was modest, and this result was consistent with an earlier result (Fig. 4.5) showing a small but significant increase in AOA of 50% acetone soluble extracts due to autoclaving. These results clearly indicate that there is an enormous difference between the effects of autoclaving on AOA of water soluble extracts of bran, compared to AOA of solvent extracts, such as 50% acetone, of the bran itself. Part of this effect is likely due to the concentration enhancement of compounds with AOA activity due to freeze-drying of the water-soluble extracts. Nevertheless, untreated bran released much lower levels of AOA related compounds compared to that in autoclaved bran.
Figure 4.21. Effects of bran autoclaving on phenolic content of free, esterified and bound phenolic fractions of bran. Averages of treatments marked by the same letter are not significantly different (p<0.05).

Figure 4.22. Effects of bran autoclaving on antioxidant activity of free, esterified and bound phenolic fractions of bran. Averages of treatments marked by the same letter are not significantly different (p<0.05).
4.6. Proximate and other chemical analysis of control and autoclaved bran, water-soluble extracts and residues.

4.6.1. Ash, mineral and phytate content

There was no significant effect of autoclaving on ash content of bran as would be expected. The average ash content of the CWRS bran was 8.6%. Likewise autoclaving compared to untreated bran produced little measurable difference in total selected mineral content of the bran (Table 4.7) which was dominated by potassium and phosphorus in this analysis; compare totals of 3.36 and 3.49% of control and autoclaved bran, respectively. For individual minerals, the difference in concentration between autoclaved and untreated bran was sometimes larger. For example the potassium content of control and autoclaved brans were 1630 and 1690 mg/100 g, respectively, a difference of 3.7%. Missing in the analysis was another major mineral, magnesium, which is typically third in concentration in bran after phosphorus and potassium.

However, for water-soluble extracts of bran, autoclaving of bran produced extracts that were higher by 45-85% in concentration of all analyzed minerals, except phosphorus. As minerals are mainly contained within bran cell walls, and within aleurone cells in particular, the expected physical disruption of cell walls by autoclaving would be expected to result in increased extractability of minerals in general. More difficult to explain are results for phosphorus which decreased by ~40% in autoclaved bran extracts (4830 vs. 2970 mg/100 g in extracts of autoclaved and control brans, respectively). Phosphorus in wheat bran is mainly associated in bound form with another bran constituent, phytic acid (inositol hexaphosphate) which also resides within the aleurone layer of bran. Accordingly, the result could be
explained by a corresponding reduction in phytate solubility due to autoclaving. Unfortunately this was not found (Fig. 4.23). The result for phosphorus needs more study.

Autoclaving of bran, both dry and wet, resulted in a considerable decrease by ~40% in phytate content analyzed for bran. On the other hand, for water soluble extracts, phytate content was markedly enhanced due to autoclaving, ~ 5- and 10-fold (from a base level of 2% in extracts of control bran) for autoclaved dry and wet bran, respectively. Evidently, wetting of wheat bran before autoclaving can produce a remarkable concentration of phytate in extracts, in this experiment, ~25% of freeze-dried material.

Figure 4.23. Phytic acid content in heat treated bran, extracts and residue samples.
<table>
<thead>
<tr>
<th>Minerals</th>
<th>Control Bran</th>
<th>Autoclaved Bran</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bran</td>
<td>Extract</td>
</tr>
<tr>
<td>Calcium</td>
<td>94.4 ± 0.4</td>
<td>91.1 ± 0.1</td>
</tr>
<tr>
<td>Copper</td>
<td>1.06 ± 0.0</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>Iron</td>
<td>14.2 ± 0.1</td>
<td>3.205 ± 0.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>1630 ± 0</td>
<td>6270 ± 70</td>
</tr>
<tr>
<td>Sodium</td>
<td>7.5 ± 0.1</td>
<td>30.9 ± 1.4</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1600 ± 14</td>
<td>4830 ± 14</td>
</tr>
<tr>
<td>Zinc</td>
<td>8.7 ± 0.1</td>
<td>2.32 ± 0.0</td>
</tr>
<tr>
<td>Total (%)</td>
<td>3.36</td>
<td>3.49</td>
</tr>
</tbody>
</table>

*Values, except for total, expressed in mg/100 g bran ± standard deviation.
4.6.2. Protein and amino acid content

Autoclaving of bran produced no significant change in protein content compared to untreated bran based on the sum of constituent amino acids (Table 4.8). On average, the protein content was 12.7%. It can be seen from the table that bran is rich in aspartic acid, glutamic acid and arginine. It has generally a fairly similar amino acid profile compared to refined flour with several exceptions. Most notably, aspartic acid and arginine content in bran are more than double that of flour, whereas glutamic acid and proline are ~44% and 50% reduced in bran.

Table 4.8. Effect of autoclaving on protein and amino acid content (%) of wheat bran, water-soluble extracts and residues*.

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Flour#</th>
<th>Control</th>
<th>Autoclave treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bran</td>
<td>Extract</td>
<td>Residue</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.51</td>
<td>1.12</td>
<td>1.74</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.34</td>
<td>0.50</td>
<td>0.57</td>
</tr>
<tr>
<td>Serine</td>
<td>0.59</td>
<td>0.61</td>
<td>0.69</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>4.57</td>
<td>2.55</td>
<td>3.10</td>
</tr>
<tr>
<td>Proline</td>
<td>1.55</td>
<td>0.76</td>
<td>1.05</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.46</td>
<td>0.85</td>
<td>0.95</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.38</td>
<td>0.76</td>
<td>0.98</td>
</tr>
<tr>
<td>Valine</td>
<td>0.54</td>
<td>0.64</td>
<td>0.75</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.19</td>
<td>0.20</td>
<td>0.21</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.47</td>
<td>0.45</td>
<td>0.55</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.89</td>
<td>0.88</td>
<td>0.95</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.22</td>
<td>0.43</td>
<td>0.51</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.64</td>
<td>0.56</td>
<td>0.58</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.27</td>
<td>0.41</td>
<td>0.43</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.32</td>
<td>0.59</td>
<td>0.64</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.46</td>
<td>1.13</td>
<td>1.22</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.32</td>
<td>0.30</td>
<td>0.55</td>
</tr>
<tr>
<td>Total Protein</td>
<td>12.74</td>
<td>12.74</td>
<td>15.47</td>
</tr>
</tbody>
</table>

*Tryptophan in bran, extracts and residues was not determined.
#Flour amino acid data from USDA Nutrient Data Base for industrial refined white flour adjusted to same protein level as for control bran.
Compared to bran, the outcome of autoclaving of bran was considerably different for the water soluble extracts. Autoclaving substantially reduced the concentration of protein and constituent amino acids in water-soluble extracts by 70% on average, from a total of ~15.5% to 5.2% in extracts of control and autoclaved bran respectively. It may be the case that amino acids have reacted with free sugars in a Maillard-type reaction, becoming insoluble in the process. This could explain the colour and aroma changes of bran noted previously due to autoclaving. The lower protein and amino acid content of the autoclaved bran extract could also be due to denaturing of protein in autoclaving conditions, resulting in protein being relatively insoluble in water.

4.6.3. Starch content

Autoclaving of bran resulted in a small reduction of bran starch content from 5.2 to 4.2% for control and autoclave dry treated bran (Fig. 4.24). The largest effect of autoclaving occurred for water soluble extracts of autoclaved dry bran; there was a reduction from 4.8% to 0.4% in water soluble extracts of control and autoclaved dry bran, respectively. By comparison, autoclaving of wet bran resulted in a much smaller difference of starch content between the corresponding water soluble extracts. However, air oven treatment of dry bran in N₂ atmosphere resulted in a considerable reduction of starch content in water soluble extracts from 4.8% to 1.7% for control and autoclaved dry bran, respectively. Evidently, dry heat treatment of bran results in reduced solubility of starch. Why autoclaving of wet bran produced no similar outcome is not known.
Figure 4.24. Effect of bran heat treatment on starch content of bran and water soluble bran extracts. “Oven N” denotes air oven treatment in N\textsubscript{2} atmosphere. Averages of different treatments marked by the same letter are not significantly different (p\textless0.05).

4.6.4 Sugar content

Simple sugar contents of fractions are reported in Table 4.9. Untreated and autoclaved bran had simple sugar levels \textless3\% which is typical. Extracting bran with water resulted in substantial removal of these sugars as observed by low values for residue fractions of control and autoclave treated bran. For the latter material, no sugars were detected. The water extracts contained significant levels of sugars. In contrast to most other analytes, water soluble extracts of autoclaved bran had much lower levels of sugars (9.8\%) compared to untreated bran extracts (18.9\%). The composition of sugars in the extract material was also very different depending on treatment. Almost all the sugar
content in extracts of autoclaved bran was due to sucrose. In contrast, water soluble extracts of untreated bran was mainly comprised of fructose and glucose in equal proportions.

Table 4.9. Effect of autoclaving of bran on the sugar content (%) of bran, water soluble extracts and residues.

<table>
<thead>
<tr>
<th></th>
<th>Control Bran</th>
<th>Control Extract</th>
<th>Control Residue</th>
<th>Autoclave Treated Bran</th>
<th>Autoclave Treated Extract</th>
<th>Autoclave Treated Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>1.48</td>
<td>0.167</td>
<td>&lt; 0.1</td>
<td>1.92</td>
<td>8.32</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.416</td>
<td>8.84</td>
<td>0.26</td>
<td>0.216</td>
<td>0.915</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.452</td>
<td>8.22</td>
<td>0.25</td>
<td>&lt; 0.1</td>
<td>0.206</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.136</td>
<td>1.69</td>
<td>&lt; 0.1</td>
<td>0.136</td>
<td>0.385</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>2.48</td>
<td>18.9</td>
<td>0.508</td>
<td>2.14</td>
<td>9.83</td>
<td>0.00</td>
</tr>
</tbody>
</table>

4.6.5. Enzyme activity

Autoclaving of dry or wet bran, but not air-ooven treatment of wet or dry bran, resulted in very substantial reduction in water-soluble extracts of polyphenol oxidase (Fig. 4.25) and peroxidase in particular (Fig. 4.26) whose activity was completely eliminated. High temperature (121 °C) treatment alone in the air oven had no effect to deactivate polyphenol oxidase (Fig. 4.25), and had only a small but significant effect to reduce the activity of peroxidase (Fig. 4.26) which evidently is more labile to high temperature conditions. Clearly, autoclaving conditions, which include elevated pressure to ~ two atmospheres during the sterilization phase, absence of oxygen and rapid depressurization prior to drying, are extremely effective to deactivate enzymes in bran.
Figure 4.25: Effect of water on peroxidase activity of different heat treatments of bran on polyphehoxidase activity.

Figure 4.26: Effect of different heat treatments on peroxidase activity.
4.6.6. B Vitamin content

Autoclave treatment of bran resulted, with one exception, in enhanced solubility of B vitamins in water solubilized extracts of bran (Table 4.10). Relative changes in the solubility of the B vitamins was as follows:

- Niacin (Vitamin B3), 78% increase
- Thiamine (Vitamin B1), 87% decrease
- Folic Acid (Vitamin B9), 22% increase
- Pantothenic Acid (Vitamin B5), 19% increase
- Pyridoxine (Vitamin B6), 35% increase

The increase in extraction of B vitamins in water soluble extracts of autoclaved bran is most likely due to the same mechanism resulting in above noted increases in solubility of other bran constituents and in increased AOA of extracts. As B vitamins are concentrated in wheat bran tissues, physical and/or chemical disruption of bran tissues and cell walls would be expected to accommodate enhanced extractability. Regarding thiamine, its substantial decrease in water soluble extracts is most likely due to its well known heat instability (Bendix et al 1951). Most notable perhaps is the remarkable increase in water extractability of niacin from autoclaved bran. Interestingly the standard AOAC International method for niacin determination (AOAC 961.14) incorporates a digestion procedure for cereal products involves autoclaving of aqueous sample suspensions at 121 °C for 2 h, to free endogenous niacin from protein, and to convert added nicotinamide to niacin (LaCroix et al 2001).
Table 4.10. Effect of autoclaving of bran on B vitamin content of bran, water soluble extracts and residues.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Autoclave Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bran</td>
<td>Extract</td>
</tr>
<tr>
<td>Niacin (Vitamin B3) mg/100g</td>
<td>29.38 ± 0.16</td>
<td>67.015 ± 2.9</td>
</tr>
<tr>
<td>Thiamine (Vitamin B1) mg/100g</td>
<td>0.695 ± 0.04</td>
<td>1.405 ± 0.02</td>
</tr>
<tr>
<td>Folic Acid (Vitamin B9) µg/100 g</td>
<td>64.06 ± 4.7</td>
<td>159.68 ± 4.7</td>
</tr>
<tr>
<td>Pantothenic Acid (Vitamin B5) mg/100g</td>
<td>2.929</td>
<td>12.68</td>
</tr>
<tr>
<td>Pyridoxine (Vitamin B6) mg/100g</td>
<td>1.389</td>
<td>5.196</td>
</tr>
</tbody>
</table>
4.6.7. Nonstarch polysaccharide and fibre content

4.6.7.1. \(\beta\)-glucan and arabinoxylan content

Wheat bran typically has low levels of \(\beta\)-glucan (Henry 1985) which was confirmed (Fig. 4.27); wheat bran had \(\sim\) 2.5% content. Autoclaving of bran, but not air-oven heat treatment of bran in \(N_2\) atmosphere, resulted in very considerable enhancement of the water extractability of \(\beta\)-glucan compared to untreated bran. For the latter samples and extracts from air-oven treated bran, \(\beta\)-glucan level was 0.1%. In contrast \(\beta\)-glucan contents of water-soluble autoclaved dry and autoclaved wet bran were 2.7% and 2.8%, respectively.

A very similar pattern of results was obtained for arabinoxylan contents of wheat bran and water soluble extracts (Fig. 4.28). Arabinoxylan content of control wheat bran was 28% and was little affected by heat treatment. As with results for \(\beta\)-glucan, arabinoxylans were sparingly extracted from untreated bran with water (1.7%), and showed a slight increase by oven treatment under \(N_2\) (to 1.9%). In contrast autoclaving resulted in a substantial increase in the extractability of AX in water-soluble extracts, to 6.4% and 6.6% for autoclaved wet and dry bran, respectively.

This result again demonstrates that autoclaving of bran has a unique physical effect to breakdown the structure of NSP laden cell walls of bran, rendering constituent compounds, such as \(\beta\)-glucans and arabinoxylans, readily extractable with water.
Figure 4.27. β-glucan content (%) in heat treated bran and extracts. “Oven N” denotes air oven treatment in N₂ atmosphere.

Figure 4.28. Arabinoxylan content (%) in heat treated bran and extracts. “Oven N” denotes air oven treatment in N₂ atmosphere.
4.6.7.2 Fibre content

Wheat bran, autoclaved or not, is largely comprised of insoluble fibre. The method used to determine fibre (AOAC 991.43) uses a sequence of starch degrading enzymes to digest samples at high temperatures (60-100 °C) combined with specific filtrations to separate insoluble from soluble fibre, and precipitations with 95% ethanol (95% ethanol precipitates fibre, but not simple sugars and oligosaccharides). Total dietary fibre (TDF) levels at ~53% were somewhat higher than is typically reported for wheat bran (Table 4.11). The CWRS wheat bran used in this thesis research contained low levels of adhering endosperm content as reflected by starch content ~5%, which corresponds to very clean bran, hence likely contributing to the high TDF values.

<table>
<thead>
<tr>
<th></th>
<th>Bran</th>
<th>Autoclaved bran</th>
<th>Bran extract</th>
<th>Autoclaved bran extract</th>
<th>Bran residue</th>
<th>Autoclaved bran residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insoluble fiber</td>
<td>49.35</td>
<td>48.15</td>
<td>0.35</td>
<td>1.60</td>
<td>72.75</td>
<td>62.45</td>
</tr>
<tr>
<td>Soluble fiber</td>
<td>3.70</td>
<td>4.35</td>
<td>3.65</td>
<td>11.65</td>
<td>2.00</td>
<td>3.45</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>53.05</td>
<td>52.50</td>
<td>4.00</td>
<td>13.25</td>
<td>74.75</td>
<td>65.90</td>
</tr>
<tr>
<td>Resistant oligosaccharides</td>
<td>3.75</td>
<td>3.75</td>
<td>8.80</td>
<td>22.70</td>
<td>0.10</td>
<td>0.25</td>
</tr>
<tr>
<td>Beta glucan</td>
<td>2.53</td>
<td>2.55</td>
<td>nd(^1)</td>
<td>2.53</td>
<td>2.66</td>
<td>2.72</td>
</tr>
<tr>
<td>Inulin</td>
<td>1.93</td>
<td>1.93</td>
<td>4.58</td>
<td>13.00</td>
<td>nd(^1)</td>
<td>nd</td>
</tr>
<tr>
<td>Total fiber(^2)</td>
<td>56.80</td>
<td>56.25</td>
<td>12.80</td>
<td>35.95</td>
<td>74.85</td>
<td>66.15</td>
</tr>
</tbody>
</table>

\(^1\)not detected; analyte level below detection limit.
\(^2\)Sum of total dietary fibre (AOAC 991.43) plus resistant oligosaccharides.

It can be seen from results in Table 4.11 that there was essentially no difference between untreated and autoclaved bran in the determination of TDF or any fibre-related component as analyzed. In contrast, very large differences in fibre contents were observed between the water-soluble extract fractions (Table 4.11). Water soluble extracts
of autoclaved bran had substantially higher TDF content, more than 300% higher than that for untreated bran; ~13% vs. 4%. Also remarkable were results for resistant oligosaccharide (RO) contents of these extract fractions, particularly for autoclaved bran (22.7%) compared to RO content in water extracts of untreated bran (8.8%). Inulin (oligosaccharides of fructose) results indicate that this fraction likely represents about 60% of RO content for autoclaved bran extracts. Considering both TDF and RO fractions, close to 36% of the composition of water-extractable compounds from autoclaved bran was fibre, ~ 300% higher than that for water extracts of untreated bran.

These results indicate that conditions of autoclaving provide an extremely effective means of enhancing the fibre solubility of wheat bran. The likely mechanism of this outcome is a combination of physical disruption of bran cell walls, thus enhancing fibre extractability combined with hydrolysis of polysaccharides to smaller and more soluble components.
5. GENERAL DISCUSSION

Whole grain can be partitioned into three major tissue components: endosperm, germ and bran. The traditional economic value of wheat resides with the most predominant fraction, i.e. the starchy endosperm which is energy-rich. Wheat endosperm, which is the source of refined wheat flour, represents ~ 82% of grain weight, but contains < 10% of the nutritive value (minerals, vitamins and dietary fibre) of whole grain (Graham et al 2009). In fact, the by-products of wheat milling, i.e. germ, and bran in particular, contain nearly all the bioactive compounds of wheat. However, despite its compelling nutritive value and functional properties for health, wheat bran is typically priced much less compared to wheat flour, and is predominantly used for animal feed.

As has been reviewed above, wheat bran contains very significant levels of dietary fibre, phenolic compounds, phytate and lesser amounts of other phytochemicals such as B vitamins including folate, lignans, sterols, and alkylresorcinols, all of which can provide health benefits to reduce the risk of many chronic diseases (Onyenho et al 1992, Zhou et al 2004, Martinez-Tome et al 2004, Liyana-Pathirana and Shahidi 2006) which is the basis for the characterization of wheat bran as a functional food. In particular, it is the antioxidant activity associated with many of the phytochemicals of wheat bran that has received considerable attention over the past decade with regard to functional properties and health benefits.

The complication of wheat bran as a functional food relates to its high fibre content, typically in the neighbourhood of 50%, which renders bran and its constituent bioactives largely insoluble and hence with limited bioavailability until passage through
the large intestine where the bran can be broken down by bacteria. Presumably, if the solubility of wheat bran could be enhanced by pre-treatment, its biological impact associated with its fibre and resident phytochemicals would also be enhanced. This was the focus of this thesis research project with autoclaving as the key pre-treatment.

A review of the literature showed that wheat grain, bran or corresponding material of other grains has been subjected to various treatments to improve its functional properties for processing (e.g. breadmaking) or to alter the solubility of various component molecules. However, a specific health related objective in these studies related to improved bioaccessibility or bioavailability of molecules was not expressed. For example, to minimize its negative effects for breadmaking, bran has been subjected to various treatments prior to its incorporation into dough such as hydration (Nelles et al 1998), fermentation (Salmenkallio-Marttila et al 2001), heat treatment in sealed vessels (de Kock et al 1999) and particle size reduction (Galliard et al 1988, Lai et al 1989b, Zhang et al 1999). Improvements due to these treatments were reported to be generally small or insignificant on processing properties such as dough mixing behaviour or loaf volume, and outcomes not well explained.

Among the many heat treatments reported in the literature on wheat bran and analogous grain products, such as roasting, boiling, steaming, microwave heating and autoclaving, it is the latter that appears to provide some of the more interesting effects, although there have been very few studies in this category, and none on wheat or wheat bran. Saulnier et al (2001) autoclaved hydrated samples of previously micronized and destarched (in boiling water with thermostabile α-amylase) maize bran and observed considerable increase in extractability of ferulic acid and arabinose and xylose in the
residue material). Saulnier et al (2001) speculated that the increase in solubility of the neutral sugars and ferulic acid upon autoclaving was due to hydrolysis of cell wall polysaccharides citing research on poplar wood (Excoffier et al 1991), or structural breakdown caused by decompression, citing thesis research done on cellulose (Debzi 1992). Both theories seem plausible. In other research, Bryngelsson et al (2002) applied autoclaving to oats and evaluated methanolic extracts for content of tocopherols, tocotrienols, avenathramides, cinnamic acids (p-coumaric, ferulic, caffeic) and vanillin. Autoclaving resulted in substantially increased levels β-tocopherol as well as ferulic acid, p-coumaric acid, and vanillin, however levels of avenathramides were reduced.

However, it was a preliminary experiment in our laboratories which applied autoclaving of bran with the objective to deactivate enzymes so as not to confound a fermentation experiment that led to this thesis project’s research on mainly autoclaving on outcomes listed in the objectives in the Introduction. In that preliminary experiment, water extracts of autoclaved bran showed 300-400% increase in antioxidant activity using a free radical scavenging assay compared to corresponding extracts from untreated bran. In addition, the emphasis on studying water extracts of bran, provides a basis to extrapolate results in terms of bioaccessibility or bioactivity, as presumably if autoclaving results in considerable enhancements in the extractability or solubility of antioxidants and soluble fibre components, then food containing autoclaved bran should also yield comparable or even greater enhancements given the nature of digestive sections which include low pH gastric conditions with pepsin, and subsequent digestion in the small intestine in moderately basic pH conditions plus pancreatin enzymes.
In fact a very recent application of in vitro digestion using wheat bran (Anson et al 2010) showed that the antioxidant capacity of wheat bran and aleurone increased substantially after 6 h of in vitro digestion modeling gastric and small intestine function. As well, bioaccessible compounds from bran (dialyzable fraction of digests) also had anti-inflammatory properties, as evidenced by the reduction in lipopolysaccharide induced TNF-α production. In a related paper (Anson et al 2009), free ferulic acid content also increased substantially during in vitro digestion, but the levels remained relatively low (<1-2%) in the dialyzable fraction (~ 50,000 MW cut off) compared to total, i.e. bound and insoluble ferulic acid. The authors concluded that “processing methods that aim at improving the bioaccessibility of ferulic acid from cereal products may be the most promising approach to expect health benefits at the systemic level.”

In this thesis research, autoclaving of bran produced many favourable outcomes which are summarized below.

Autoclaved wheat bran (using a standard cycle of 15 min sterilization and 10 min drying) was substantially improved in its aroma that was obvious by subjective evaluation. Such bran appeared to be very stable, and even after several years in uncareful storage at room temperature, the bran showed absolutely no evidence of rancidity. In contrast, untreated bran typically revealed a rancid aroma after only one or two months on the shelf. Exceptionally stable wheat bran would be highly desirable by industry, as it would also minimize the need for careful stock control and storage of whole wheat flour (Galliard 1986).
Autoclaving of white wheat bran produced a product, again evaluated subjectively, with a very appealing golden colour that may have benefits to industry in development of new whole wheat bread products to the consumer.

Autoclaving yielded bran with little to no enzyme activity as evaluated on the basis of polyphenol oxidase and peroxidase whose levels were considerable in untreated bran. This result may explain the stability of the wheat bran upon prolonged storage as presumably lipase and other enzymes that promote rancidity were not active.

Additional evidence of the lack of enzyme activity in extracts of autoclaved bran were revealed in dough mixing experiments wherein a base flour was supplemented with 15% of its weight with freeze dried water soluble extracts. For extracts from autoclaved bran, there was essentially no effect on dough mixing behaviour. In contrast, mixing doughs in the presence of water-soluble extracts of untreated bran resulted in the absence of dough formation. It was proposed that this result occurred due to the presence in the extracts of enzymes such as xylanase which are extractable in water (Van Craeyveld et al 2010) and can be effectively deactivated by normal autoclave conditions (Van Craeyveld et al 2010). McCleary (1986) has demonstrated that adding small amounts of xylanase enzyme to wheat flour dough can completely and almost immediately destroy normal dough mixing properties by hydrolysis of arabinoxylans which have significant water binding properties disproportionate to their relatively low concentration in wheat endosperm and flour (Bushuk 1966).

Freeze dried water soluble extracts of autoclaved bran had substantially enhanced levels of antioxidant activity compared to corresponding extracts from untreated bran.
The level of enhancements in free radical scavenging activities using DPPH and ABTS assays were typically 300-500% in the many different experiments that were performed.

Autoclave processing conditions in terms of number of autoclave cycles, sterilization time duration and temperature were very well characterized in terms of their effects on enhancing the antioxidant activity, as well as the colour and aroma of the bran. Interestingly, the autoclaving effect to enhance antioxidant activity (as well as bran aroma and colour as noted above) was largely manifested (~95% of enhanced antioxidant activity) after only one-third (i.e. 5 min) the duration of a standard autoclave cycle. In contrast, extending the autoclave cycle time or the number of autoclave cycles beyond the standard parameters resulted in increasingly negative sensory properties, i.e. bran became progressively darker and had a very noticeable burnt aroma.

Results seemed in accord with formation of Maillard reaction products. Previous studies with nuts and pulses (Acar et al 2009) have suggested that autoclaving causes a Maillard reaction and most Maillard reaction by-products may form new compounds which have antioxidant capacity. Evidence for Maillard reaction during autoclaving was substantially reduced content of amino acids and free sugars in autoclaved bran extracts.

Bran colour became very dark brown after 90 min of sterilization time in the autoclave for the red-coloured CWRS wheat bran. Even for the white-branned Platte bran, colour of the product was only marginally lighter. It was observed that treating bran in the autoclave for more than 20-30 min of sterilization at 121 °C would not be acceptable for a food application due to colour and aroma. Results indicated that autoclaving bran at 121 °C for 10-15 min sterilization with a drying cycle was close to
ideal to obtain desired bran colour and aroma together with increased solubilisation of antioxidant compounds.

Freeze dried water soluble extracts of autoclaved bran had significantly enhanced levels of phenolic content compared to that of untreated bran. However there was no close correspondence between AOA and total phenolic content. The autoclave effect was much greater to enhance extraction of antioxidant compounds with free radical scavenging properties compared to phenolic compounds evaluated by the Folin-Ciocalteu method. As observed previously, the Folin method may not accurately estimate phenolic compounds and/or compounds with AOA extractable in water may not all be phenolic in nature.

Among the most interesting and compelling results of bran autoclaving was the observed enhancement in water extraction of a very broad spectrum of bran bioactive constituents including minerals, phytate, B vitamins, and non-starch polysaccharide fibre compounds suggesting enhanced bioaccessibility if not bioavailability of these phytochemicals. Compared to water soluble extracts of untreated bran, autoclaved bran produced extracts that were higher in mineral content (except phosphorus) by 45-85%. Phytate content was markedly enhanced due to autoclaving from a base level of ~ 2% in extracts of control bran to 10% and 25% in extracts of autoclaved dry and wet bran, respectively. Autoclaving wet bran appears to be a novel approach to substantially enhance the water extractability/solubility of phytic acid, and may accommodate a new protocol for isolation and purification of phytic acid for commercial use.

Phytic acid has traditionally been considered to be an antinutrient (most notably iron deficiency anemia) associated with its ability to bind divalent cations and reduce
their bioavailability for people consuming very narrow diet of predominantly whole
grains. However, antioxidant activity has also been ascribed to phytic acid or inositol
hexaphosphate (Graf et al 1987, Graf and Eaton 1990, Martinez-Tome et al 2004) which
is known to have potent cancer preventive properties (Midorikawa et al 2001,
Somasundar et al 2005).

Except for thiamine, autoclave treatment of bran resulted in significant increases
in extracted B vitamins, ranging from 22% (B9 folic acid) to 78% (B3 niacin) relative to
corresponding extracts from untreated bran.

For β-glucans which were sparingly soluble in water extracts from untreated bran,
levels in extracts of autoclaved bran increased to 2.7%. Likewise for arabinobioxylan
content of extracts, concentration increased from 1.7% to 6.6% in untreated and
autoclaved bran, respectively. Mirroring these results were similar outcomes for total
dietary fibre and resistant oligosaccharides which increased in water soluble extracts of
bran due to autoclaving, from 4% to 13.2%, and 8.8% to 22.7%, respectively.
6. CONCLUSIONS

Autoclaving of dry bran was shown to be extremely effective to enhance the solubility of wheat bran and constituent phytochemical compounds. The likely mechanism which remains to be proven is a combination of physical disruption of bran cell walls, thus enhancing fibre extractability, combined with hydrolysis of polysaccharides to smaller and more soluble components. As the phenolic compound, ferulic acid is a molecular component of arabinoxylans, the process also results, fortuitously, in enhanced levels of antioxidant activity in extracts, which could also be further enhanced by Maillard reaction products. Presumably the standard autoclave procedure combining high temperature, elevated pressure, an oxygen free atmosphere, and rapid depressurization towards the end of the cycle together contribute a unique and potent physicochemical effect to breakdown the structure of non-starch polysaccharide laden cell walls of bran, rendering constituent bioactive compounds readily extractable with water.

Results taken together were very compelling. They point to the development of novel wheat bran and extracts with considerable commercial potential for whole grain food use and a variety of nutraceutical applications. The autoclaving treatment that produces this novel bran likely represents a highly significant development in bran processing technology that has the potential to add substantial value to common wheat bran for food and health applications far beyond what may be possible with whole grain wheat or normal bran in conventional foods. That autoclaving is eminently scalable from microbiological and medical lab-based sterilization, to 20 metre long units to cure
composite jet airplane fuselages and other components, indicates that there are no technological barriers for commercialization in the grain food industry.

Perhaps the most important outcome of the research described in this thesis is that the nutritional and functional food benefits of normal wheat bran probably represent a fraction of its potential due to the limited digestibility of wheat bran, which in turn, limits the bioavailability of its fiber and constituent bioactive compounds. Autoclaved bran appears to have no similar limitation.

Recommended future research to better understand the nature of the autoclaving effect should include microscopy studies to potentially reveal structural changes in bran cell walls, comprehensive chemical characterization of phenolic and other compounds that appear to contribute to enhancement of antioxidant activity of autoclaved bran extracts, and a wide range of pre-clinical and clinical studies on the efficacy of the novel bran and extracts to reduce the risk of chronic disease.
7. REFERENCES


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Inhibition of low-density lipoprotein oxidation and oxygen radical absorbance capacity. Journal of Agricultural and Food Chemistry 47: 4888-4893.


Norton, T. and Sun, D.-W. 2008. Recent advances in the use of high pressure as an effective processing technique in the food industry. Food Bioprocess Technol. 1:2–34


Figure A.1. Effect of bran container type on antioxidant activity (DPPH free radical % decolourization) of water-soluble bran extracts of untreated and autoclaved bran. The glass bottles had dimensions 4 x 4 x 14 cm in height with a volume capacity of 190 ml. The bottles were filled with bran to a height of 5 cm and took up ~ 90 ml of bottle volume. The bottles were loosely capped during the treatment. The disposable aluminum trays had dimensions 7 x 18 x 5 cm in height. The depth of the wheat bran in each aluminum tray was 0.8 cm. The cross sectional area of the bran surface was ~ 126 cm². The effect of container on AOA of bran extracts was negligible.
Table A.1. Effect of autoclaving of bran on the sugar content (%) of bran, water soluble extracts and residues.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Autoclave Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bran</td>
<td>Extract</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.48</td>
<td>0.167</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.416</td>
<td>8.84</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.452</td>
<td>8.22</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.136</td>
<td>1.69</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>2.48</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Simple sugar contents of fractions are reported in Table A.1. Untreated and autoclaved bran had simple sugar levels < 3% which is typical. Extracting bran with water resulted in substantial removal of these sugars as observed by low values for residue fractions of control and autoclave treated bran. The water extracts contained significant levels of sugars. In contrast to most other analytes, water soluble extracts of autoclaved bran had much lower levels of sugars (9.8%) compared to untreated bran extracts (18.9%). The composition of sugars in the extract material was also very different depending on treatment. Almost all the sugar content in extracts of autoclaved bran was due to sucrose. In contrast, water soluble extracts of untreated bran was mainly comprised of fructose and glucose in equal proportions.
Table A.2. Summary of composition of control and autoclave treated bran, water-soluble extracts and residues.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Autoclave Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bran</td>
<td>Extract</td>
</tr>
<tr>
<td>Moisture</td>
<td>8.65</td>
<td>12.93</td>
</tr>
<tr>
<td>Total amino acids</td>
<td>12.73</td>
<td>15.44</td>
</tr>
<tr>
<td>Total starch</td>
<td>5.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Total sugars</td>
<td>2.48</td>
<td>18.9</td>
</tr>
<tr>
<td>Total dietary fibre</td>
<td>53.05</td>
<td>4.0</td>
</tr>
<tr>
<td>Resistant oligosaccharides</td>
<td>3.75</td>
<td>8.80</td>
</tr>
<tr>
<td>Total minerals</td>
<td>3.36</td>
<td>11.23</td>
</tr>
<tr>
<td>Phenolics (FA eqv)</td>
<td>5.46</td>
<td>11.07</td>
</tr>
<tr>
<td>Phytic acid</td>
<td>9.98</td>
<td>2.2</td>
</tr>
<tr>
<td>B Vitamins</td>
<td>0.034</td>
<td>0.086</td>
</tr>
<tr>
<td>Sum</td>
<td>104.4</td>
<td>89.5</td>
</tr>
</tbody>
</table>

nd, not detected.