# Effects of *in vitro* Gastrointestinal Digestion on Extractability of Nutrient and Bioactive Compounds from Wheat Bran

BY

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A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

In Partial Fulfillment of the Degree of

MASTER OF SCIENCE

Department of Food Science

University of Manitoba

Winnipeg, Manitoba

## ACKNOWLEDGEMENTS

I would like to take this opportunity to thank my co-advisors, Dr. Harry. Sapirstein and Dr. Ames for their guidance, patience, and encouragement they gave me during my M. Sc. Project. Thank you for providing your knowledge and experience to assist me during my graduate studies. I am thankful to my advisory committee Drs. Peter Eck and Curtis Rempel for providing valuable input and support for my thesis research.

I am grateful to the Canadian Wheat Board for allowing me to pursue my graduate studies through their financial support. Thanks to Arshala Karunasena Dona for helping with a smooth transition into graduate research, by providing technical assistance and guidance. A special thanks to Yang Qiu, Allison Ser, and Pat Kenyon of the Food Science department for the advice and training they have provided me over the years. Thank you to Dr. Aluko and his graduate students who graciously provided knowledge and time to help me conduct lab research important to my thesis project. I thank Dr. Ames and her research staff, Tracy Exley, Joanne Storsley, Natalie Middlestead, and Jonathan Leong from the Cereal Research Centre – AAFC who provided technical support that very much helped me complete my thesis research. I am thankful to Dr. Cindy Grant and Mr. Josh Price from the Brandon Research Centre of AAFC for technical assistance in mineral analysis of my thesis samples. Thank you to David Niziol and Jennifer Fehr of the Cereal Research Centre - AAFC, Winnipeg for technical assistance in the combustion nitrogen analysis of my thesis samples. Thanks also to Ashok Sarkar and Frank Bergen of CIGI for assistance with the milling of my

samples. Thank you to Rob Ellis (Department of Soil Science, U of M) who kindly loaned me a muffle furnace for ash determination. Thank you to Drs. Gary Crow (Department of Animal Science, U of M) and Francis Zvomuya (Department of Soil Science, U of M) for statistical analysis advice provided. Thank you to all the technicians of the Richardson Centre for Functional Foods and Nutraceuticals and to all my fellow graduate students from the Food Science department for their support.

To my family, I thank you for your unwavering support throughout my academic years. The assistance you all have provided me I consider to be invaluable.

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## ABSTRACT

A static *in vitro* digestion method was developed as a strategy to understand the effects of modelled digestion on the solubility of nutrients and selected bioactive compounds of autoclaved and untreated wheat bran. Brans from common soft, hard, and durum wheats were evaluated. Results indicated that the *in vitro* digestion protocol was suitable and effective. Effects of gastrointestinal simulation were considerably larger than gastric digestion alone. Digestibility of the brans ranged from 28.1 to 47.9%. Digestibility of bran minerals, starch and protein was substantial compared to lesser, but still significant, effects on fibre. Fibre solubility was significantly enhanced due to autoclaving. Total phenolic content, free radical scavenging and metal chelation activity were all substantially increased in soluble digests. Yields of these factors indicated that digestion of wheat bran releases ample levels of antioxidants that would be available for absorption in the small intestine to promote beneficial health effects.

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# 1. INTRODUCTION

Wheat bran with its high fibre and low endosperm content is traditionally viewed as a low value by-product of milling, with most being used for animal feed and sold at substantial discount compared to refined wheat flour. On the other hand, refined wheat flour is energy rich due to its high starch concentration derived from endosperm which also contains gluten proteins. The latter gives wheat flour its unique ability to form viscoelastic doughs and confers its diverse food functionality for making bread and numerous other leavened and unleavened products. While wheat bran is energy poor and lacks the processing functionality of refined wheat flour, it is a very compelling grain fraction as it contains nearly all the biologically active compounds of whole wheat. Some are in the germ, most notably flavonoids and tocopherols, but by far, the greater majority of bioactive compounds are in the bran fraction (Liu, 2007). The term "bioactive" refers to any non-nutrient compound in bran that is potentially bioavailable and biologically active. Wheat bran has very high levels of phenolic compounds and other phytochemicals, and combined with its high content of dietary fibre (mostly arabinoxylans (AX) and cellulose with much lesser amounts of β-glucan, especially compared to barley), can provide considerable nutritional and health benefits to consumers (Martinez-Tome et al. 2004).

Due to wheat bran's distinct makeup of bioactive components including dietary fibre, consumption of whole wheat has been shown consistently to contribute to reduced risk of heart disease, gastrointestinal cancer, and type 2 diabetes (Moore et al. 2006). Phenolic compounds in particular, which are ubiquitous in the plant kingdom, derive

their importance in chronic disease risk reduction stemming at least in part from the prevention or mitigation of oxidative damage to biological molecules including proteins, DNA and lipids (Moore et al. 2006). That damage is also associated with complex inflammatory responses at the cellular level which may be the root cause of many chronic diseases. Accordingly, the antioxidant properties of many phenolic compounds, including free radical scavenging and lessening of inflammatory responses, is the likely reason why consumption of whole grain and bran is effective in promoting health and lowering risk of many diseases.

While much is known about the content, composition, and antioxidant activity of bioactive compounds of wheat and other cereal grains, there is relatively little knowledge about aspects related to their bioavailability. The main reasons relate to the practical challenges and biochemical complexities of this kind of research which involves clinical or *in vivo* experiments and access to biological fluids and tissues. While it is the domain of medical research to provide the ultimate answers to questions of bioavailability and efficacy of plant bioactive compounds for human health, there remain many questions about extractability and bioaccessibility that can be answered by chemical analytical methodology performed *in vitro*. In particular, simulated digestion, involving chemistry mimicking physiological conditions present in the human gastrointestinal (GI) tract, offers a very useful strategy to developing an understanding of the composition and concentration of bioactive components from wheat bran that would be potentially present for absorption *in vivo*. There has been very little of this sort of research carried out to date particularly for cereal grain material. A very recent application of this methodology (Anson et al. 2010) showed that the antioxidant

capacity of wheat bran and aleurone increased substantially after 6 hr of *in vitro* digestion modeling gastric (GA) and small intestine (SI) 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. (2009a) reported that free ferulic acid (FA) content (by differential solvent fractionation) also increased substantially during *in vitro* digestion, but the levels remained relatively very low (<1-2%) in the dialyzable fraction (~ 50,000 MW cut off) compared to total for bran and flour, i.e. bound and insoluble FA. No other phenolic compounds or other bioactive constituents of bran were studied in these papers. The authors concluded that "processing methods that aim at improving the bioaccessibility of FA from cereal products may be the most promising approach to expect health benefits at the systemic level."

The statement above suggests that the nutritional and functional food benefits of normal wheat bran could represent a fraction of its full potential due to its limited (fibre) solubility which in turns limits the bioavailability of constituent bioactive compounds. Regardless of the nature of the bran, it would be expected that, compared to water as a solvent, the extractability of bran bioactive compounds such as phenolic antioxidants and low molecular weight soluble fibre should be enhanced considerably by subjecting the bran to aqueous conditions that simulate the gastric and/or upper GI tract, including mild acid hydrolysis and the presence of enzymes such as pepsin and pancreatin, which is the subject of this thesis research.

The long-term goal of this research is to improve the understanding of the functional properties of wheat bran in order to maximize the economic value of the bran

as well as that of the entire grain. Another long term goal is to promote healthier food consumption by increased usage of foods enriched with whole wheat or bran fractions in which bioactive compounds are concentrated. The broad aims of the research were 1) to develop a suitable static model of *in vitro* digestion for wheat bran material, and 2) to develop an understanding of effects of modelled digestion on the solubility of wheat bran fibre and bioactives, i.e. to what extent is wheat bran digestibility altered.

The specific objectives of the research are as follows:

- Determine if autoclaving has an advantage compared to untreated wheat bran in extractability and potential bioactivity of compounds arising from *in vitro* digestion of bran
- Determine the separate contributions of gastric and small intestine conditions to analytical outcomes for a selection of nutrients, fibre constituents and antioxidant-related factors
- Determine if analytical outcomes of *in vitro* digestion are significantly affected by the type of wheat bran (essentially wheat genotype in this study, i.e. durum (tetraploid) wheat, hard red spring common (hexaploid) wheat, soft white spring common wheat)

# 2. LITERATURE REVIEW

## 2.1. Wheat classes

Wheat belongs to the Triticum genus of the Gramineae family. Classes of wheat are categorized based on agronomic criteria such as kernel hardness and colour. Kernel hardness is categorized as either hard or soft kernel. Hard kernels produce coarser flour when milled than soft kernels due their strong starch granule-protein matrix (Smith, 1995). Kernel hardness and protein content are the main factors in deciding what type of wheat will be used to produce a food product. Kernel colour, hence bran color, for hexaploid common wheat is generally classified as red or white, while amber is the typical descriptor of the colour of tetraploid durum wheat. A practical and perhaps the only noticeable difference between red and white common wheat occurs during milling. For white wheat, millers can be less strict when removing bran fragments to improve flour yield and still retain good flour brightness and colour (Smith, 1995). Also, white wheats generally yield a color advantage in finished products like noodles and tortillas (Ambalamaatil et al. 2006). Durum wheat possesses a harder kernel compared with hard common wheat, and has a distinct amber colour. Wheat can also be classified in terms of its growing habit, like spring wheat and winter wheat. Commercial classes of wheat produced in Canada include Canada Western Red Spring (CWRS), Canada Western Amber Durum (CWAD), Canada Western Extra Strong (CWES), Canada Prairie Spring Red (CPSR), Canada Western Red Winter (CWRW), Canada Prairie Spring White (CPSW), and Canada Western Soft White Spring (CWSWS). The current worldwide wheat production consists of 95% hexaploid bread wheat and 5% tetraploid

durum wheat (Shewry, 2009). This thesis project includes samples of wheat bran from CWAD, CWRS, and CWSWS classes.

## 2.2. Wheat kernel structure and composition

All cereal grains are made up of three major parts, endosperm, bran (pericarp, testa and aleurone) and germ (embryo and scutellum) (Fig. 2.1). Germ contains the plant embryo or seed, while the endosperm provides energy or food for the growing seed. The function of bran is to protect the grain from microorganisms and insects and from adverse weather conditions (Richardson, 2006). A crease running parallel to wheat kernel's long axis on its ventral side covers ~ 25% of the kernel surface and presents a problem when milling (Posner, 2000; Evers and Millart, 2002) as bran in the crease cannot be effectively removed. Low extraction yields would be required to produce refined flour free of bran contamination or vice versa. In conventional milling, the major kernel fractions are well separated, but with some contamination of the complimentary fractions. Whole grain wheat flour and products contain the full complement of endosperm, bran and germ.

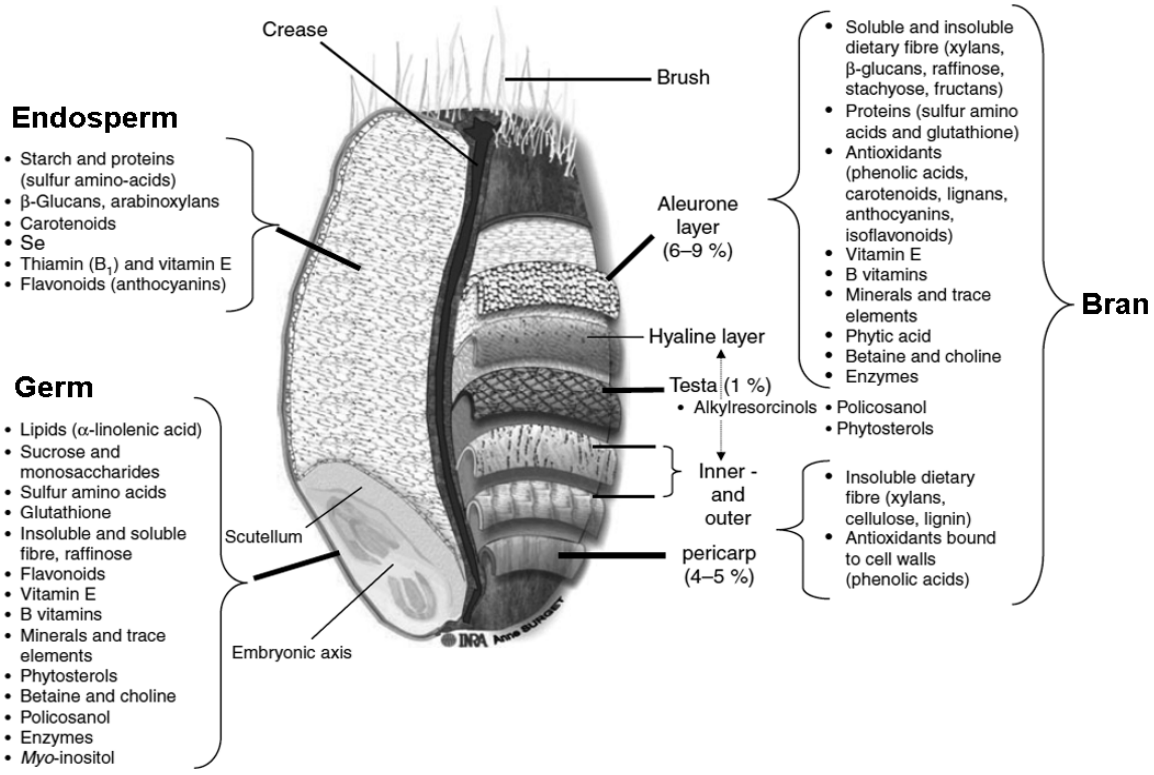


Figure 2.1. Wheat kernel with main nutrient and bioactive compounds in tissue fractions. Adapted from Fardet (2010).

The typical proportions of the endosperm, bran, and germ from a milled wheat kernel are 82-33, 14-15, and 2-3%, respectively (Belitz et al. 2009). In descending order of content, whole wheat is composed of carbohydrates, protein, lipid, minerals or ash (Table 2.1), and a phytochemical portion which, quantitatively, is in relatively low concentration (< 2%). Most of the carbohydrates are polysaccharides comprising, starch, and non-starch polysaccharides, i.e. arabinoxylans and cellulose. The latter two components form the bulk of the fibre fraction of whole wheat.

Table 2.1. Proximate composition of different layers of whole wheat grain[a,b]

| Nutrients | Whole wheat (%) | Refined flour (endosperm %) | Wheat bran (%) | Wheat germ (%) |
|---|---|---|---|---|
| Moisture | 10.3 | 11.9 | 9.9 | 11.1 |
| Protein | 13.7 | 10.3 | 15.6 | 23.2 |
| Total lipid | 1.9 | 0.98 | 4.3 | 9.7 |
| Ash (minerals) | 1.6 | 0.47 | 5.8 | 4.2 |
| Carbohydrates | 72.6 | 76.3 | 64.5 | 51.8 |
| Total dietary fibre | 12.2 | 2.7 | 47.8 | 13.2 |
| Sugars | 0.41 | 0.27 | 0.41 | 0 |
| Vitamins | 0.11 | 0.01 | 0.09 | 0.01 |
| Phytochemicals[b] | < 2.0 | | | |

[a] Data source: USDA National nutrient data base for standard reference (U.S. Department of Agriculture, 2008).
[b] Refer to Table 2.2.

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, germ also has significant levels of mineral content compared to endosperm. The majority of vitamins are concentrated in bran. 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 more than three times the fibre contained in wheat germ. Dietary fibre is clearly a major bioactive component of bran that contributes the substantial health functionality ascribed to whole wheat.

## 2.3. Wheat bran structure and composition

The most complex tissue in wheat is bran as it contains the outer and inner pericarp (fruit coat), nucellar epidermis (hyaline later, perisperm), seed coat (testa, spermoderm, integument), and aleurone layer (botanically part of endosperm but removed together with the bran during milling) (Fig. 2.1). In wheat milling, bran has been traditionally viewed as a low value by-product, with most being used for animal feed. Bran in the commercial sense is not the same as the botanical form. Commercial bran is produced at various stages of milling that creates varying levels of particle size and endosperm content (Posner, 2000, Dexter and Sarkar, 2004). Common practice when using cereals for food since the ancient times was to produce ground flour and semolina for bread and pasta products. Bran by-products would traditionally be obtained from a roller milling process. Roller milling consists of a series of corrugated and smooth rolls that systematically grinds and reduces the endosperm to flour particles while also separating bran, germ, and foreign material by using purification systems (Dexter and Sarkar, 2004). Another more recent development in bran recovery from the wheat kernel is a process called debranning. Debranning sequentially removes the outer layer of cereal kernels in a controlled way by abrasion (pearling) and/or friction (peeling) (Hemery et al. 2007). Pearling involves the rubbing of kernels against an abrasive stone to remove outer layers of a kernel. Peeling consists of kernels rubbing against each other while passing through a machine to remove the peripheral layers. Debranning was initially developed to reduce microbial, heavy metal, and sand contamination coming from the outer layers of kernels but has also been shown to improve flour refinement as well (Mousia et al. 2004). Removing large amounts of bran

at the debranning stage leaves less bran for milling, which reduces the amount of bran contamination in flour streams. Examples of bran products currently on the market that are used as a dietary fibre supplement include wheat bran alone, wheat bran powder, wheat bran with malt flavour, bran breakfast cereal, and tablet of bran (Martinez-Tome et al. 2004).

The average proportions by weight of the outer pericarp, inner pericarp, seed coat plus nucellar layer, and aleurone layer is 4.3, 1.4, 2.2, and 6.7-7.0%, respectively (MacMasters et al. 1971). The thickness of bran layer is known to vary from the thick aleurone layer (up to 65 μm), thinner outer pericarp (15-30 μm), and the most thin layer of the seed coat (5-8 μm) (Barron et al. 2007). The aleurone layer compared to the pericarp fraction of bran has higher concentrations of protein, minerals, vitamins (MacMasters et al. 1971), β-glucans and ferulic acid (Antoine et al. 2003, Harris et al. 2005).

Whole wheat is rich in bioactive compounds, but wheat bran is even richer. Table 2.2 presents a detailed comparison of the composition of fibre and fibre constituents and functional components in whole wheat and bran. Wheat germ and bran in particular contains the majority of bioactives in the grain. The bioactive constituents of bran encompass the total fibre fraction which includes AX as wheat's principle non-starch polysaccharide (NSP) (Martinez-Tome et al. 2004). Wheat bran also contains significant quantities of oligosaccharides such as fructans and inulin. In addition to minerals and B vitamins (not shown in Table 2.2), wheat bran also enhances whole wheat bioactive composition in terms of phenolic compounds (especially phenolic acids), phytic acid, sterols, betaine, and alkylresorcinols; the latter three compounds

being present in mg/g concentrations, whereas choline, flavonoids, carotenoids, and lignans are present in lower concentrations.

Table 2.2. Composition of phytochemicals in whole wheat and bran

| Phytochemicals | Whole grain | Bran |
|---|---|---|
| Fibre | | |
| β-glucan (%) | 0.4-1.4[1] | 0.4 [2] |
| Arabinoxylan (%) | 3.75-8.30[3] | 8.9-28.0[4,5] |
| Inulin /fructan (%) | 2.3[6] | 3.4-4.4[6,7] |
| Cellulose (%) | 1.64[8], 2.4[4] | 32.2[9] |
| Lignin (%) | 1.40-3.25[4] | 5.2[9], 3.5[10] |
| Lignan (µg/g) | 3.40 -22.7[11] | 110[12] |
| | 2.67-7.11[13] | |
| Phenolic acids (µg/g) Total | 160-1020[14], 1342[15] | 7610-13,840[14] |
| Ferulic | 640 - 1270[16] | 1942-5400[17] |
| | 160-1020[15] | 1380-6310[15] |
| Sinapic | 1.3-63[17] | 170-300[17] |
| ρ-coumaric | 0.2-37.2[17] | 100-457[17] |
| Syringic | 13[15] | 32[15] |
| Vanillic | 0.6-35[17] | 100-164[17] |
| ρ-hydroxybenzoic acid | 7.4[15] | 22[15] |
| Alkylresorcinols (µg/g) | 489-1100[18] | 2672[18] |
| | 227-639[19] | 2211[19],4000[20] |
| Sterols (mg/g) | 5.9-6.421, 7.2-8.3[22] | 15.0-16.7[21], 16.8-18.5[22] |
| Carotenoids (µg/g) | 1.48-2.71[23] | 0.68-3.80[24], 1.41-2.18[25] |
| Zeaxanthin | 0.25-0.53[23] | 0.25-0.40[25]-2.19[24] |
| Lutein | 0.67-2.11[23] | 0.50-1.80[24] |
| β-cryptoxanthin | 0.12-0.19[23] | 0.12[25]-0.64[24] |
| β-carotene | 0.18-0.36[23] | 0.03[25] – 0.18[24] |
| Tocopherol (µg/g) | | |
| Tocopherol | 23.0[26], 16.2[27] | 8.3[27], 3.8-22.7[24] |
| Tocotrienol | 37.1[26], 28.4[27] | 31.8[27] |
| Betaine (mg/g) | 2.91[28] | 12.93[28], 13.39[29], 8.67[30] |
| Choline (mg/g) | 0.27[28] | 0.88[28], 0.74[29], 1.02[30] |
| Phytic acid (%) | 0.53-1.08[31], 1.23-2.23[32] | 4.24 – 6.12[32] |
| Flavonoids (µg/g) | 307-432[33], 263-575[34] | 149-406[35] |
| | 210est[36], 96-1030[37] | 568[36] |

Data sources:

1. Izydorczyk and Dexter (2008)
2. Wood et al. (2002)
3. Pritchard et al. (2011)
4. Gebruers et al. (2008)
5. Wang et al. (2006)
19. Chen et al. (2004)
20. Landberg et al. (2008)
21. Hakala et al. (2002)
22. Piironen et al. (2002)
23. Okarter et al. (2010)

6. Zhou et al. (2005)
7. Karppinen et al. (2000)
8. Englyst et al. (1982)
9. Claye et al. (1996)
10. Chen et al. (1998)
11. Smeds et al. (2009)
11. Piironen et al. (2002)
12. Mazur and Adlercreutz (1998)
13. Dinelli et al. (2007)
14. Fardet (2010)
15. Mattila et al. (2005)
16. Zhao and Moghadasian 2008)
17. Vitaglione et al. (2008)
18. Ross et al. (2003)
24. Zhou et al. (2004)
25. Zhou et al. (2005)
26. Nielsen and Hansen (2008)
27. Engelsen and Hansen (2009)
28. Likes et al. (2007)
29. Zeisel et al. (2003)
30. Graham et al. (2009)
31. Febles et al. (2002)
32. Anjum et al. (2002)
33. Adom et al. (2003)
34. Asenstorfer et al. (2006)
35. Feng and McDonald (1989)
36. Hung et al. (2010)
37. Liu et al. (2010), includes purple wheat

Virtually all these bioactive compounds have been linked to positive health efficacies (Marquardt et al. 2007; Fardet, 2010; Okarter and Liu, 2010; Jonnalagadda et al. 2011), as discussed later.

Phenolics are a very noteworthy constituent of wheat bran. Apart from flavonoids which are found to a large extent in wheat germ (King 1962, Asenstorfer et al. 2006) and in relatively low concentration in bran, virtually all other phenolic compounds (phenolic acids, phenolic acid esters) reside in wheat bran tissues, most notably aleurone. 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 pathogenic fungi and other microorganisms (Fulcher et al. 1972, McKeehen et al. 1999). Cross-linked arabinoxylans exist due to oxidative coupling of FA esters in plant cell walls via peroxidase action, creating di-FAs (Garcia-Conesea et al. 1999).

Phenolics acids in general can be subdivided into two major chemical groupings; hydroxybenzoic acids and hydroxycinnamic acids. Hydroxybenzoic acids include p-hydroxybenzoic, protocatechuic, vanillic acid, synergic 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. The concentration of hydroxybenzoic acids in wheat grain is low compared to hydroxycinnamic acids, and only p-hydroxybenzoic acid has been reported in soluble extracts of wheat bran (Li et al. 2005, Zhou et al. 2005) with levels of ~ 9-20 μg/g bran. The more predominant hydroxycinnamic acids include ferulic, ρ-coumaric, caffeic and syringic acids which form links to cell wall structural components such as pentosans, cellulose and lignins through ester bonds (Liu, 2007).

By far, the predominant phenolic acid in wheat is (trans) FA which was found to comprise 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. Wheat bran is one of the richest sources of FA with levels as high as ~ 1400 mg/ 100g (Zhao and Moghadasian, 2008).

Like all phenolic acids in wheat, FA exists as free, esterified and bound forms (Hatcher and Kruger, 1997, Liyana-Pathirana and Shahidi, 2006). However, it is mainly esterified to arabinoxylan polymers at the C(0)-5 position of arabinosyl side-chains (Kern et al. 2003; Yuan et al. 2005) which contributes to its insolubility in organic solvents and structural properties of wheat bran tissues noted previously. Insoluble bound FA is present in significantly greater amounts compared to free and soluble-

conjugated FA in wheat (Labat et al. 2000, Adom et al. 2005). In the insoluble bound fraction of total phenolics, FA was essentially the only detectable phenolic acid (Sosulski et al. 1982; Hatcher and Kruger, 1997). Adom and Liu (2002) reported FA distribution in free, soluble conjugate, and bound forms as 0.2 %, 1 %, and 98.8 %, respectively

FA is predominant in wheat aleurone, pericarp and embryo cells, while trace amount can be found in starchy endosperm (Sosulski et al. 1982; Liu, 2007). Saulnier et al. (2007) and Anson et al. (2008) studied the distribution of FA in different hand-dissected fractions of whole wheat, especially different layers of bran. Aleurone cells were clearly associated with the highest concentration of FA (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 FA, much higher than those in the pericarp. Total FA concentration in what bran has been reported to be four times higher than that of whole wheat grain (Yu and Cheng, 2008). FA content also significantly differs between cultivars and different growing locations of wheat varieties (Abdel-Aal et al. 2001; Adom et al. 2003).

Perhaps the most notable phytochemical constituent of wheat bran is phytic acid which is composed of a simple sugar (inositol) with six phosphate groups attached to each carbon. It is typically found in food sources high in fibre content (Shamsuddin 2002, Somasundar et al. 2005). Phytate refers to the magnesium or calcium salts of phytic acid and represents the main storage form of phosphorus in cereal grains. Interestingly, phytic acid represents the most highly concentrated phytochemical in

wheat and wheat bran in 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. By contrast endosperm tissue of wheat contains very little phytic acid.

Additional discussion of this and other bran constituents, e.g. fibre components (cellulose, β-glucan, AX, lignin, fructan), lignans, alkylresorcinols, and phytosterols) is presented later in this literature review.

## 2.4. Antioxidant activity of wheat bran components

Phytochemicals (e.g. phenolic acids, carotenoids, tocopherols, flavonoids) provide the majority of the antioxidant activities of foods (Tsao, 2008). Antioxidants are a group of small molecular weight phytochemicals 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 strong antioxidant activities. The majority of phytochemicals present in wheat bran are phenolic compounds which make up more than 60% of the total (Table 2.2). There have been a great many published reports of antioxidant activity (AOA) of wheat, wheat bran and fractions. Some studies focused on the AOA of wheat milling fractions (Adom et al. 2005, Beta et al. 2005, Gallardo et al. 2006, Wang et al. 2006, Liyana-Pathirana and Shahidi, 2007), while others measured antioxidant activity of wheat bran specifically using few different antioxidant assays (Iqbal et al. 2007, Verma et al. 2008, Martinez-

Tome et al. 2004). There have also been a few studies have of genotype and environment effects on AOA of wheat bran (Mpofu et al. 2005, Zhou et al. 2004).

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). 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-picryhydrazyl 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 2-3% 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. 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. As with previous studies, phenolic content and AOA

increased in a correlated manner from the innermost to outermost layers of the wheat grain.

Clearly then, antioxidants in wheat are closely associated with bran tissue and its concentration of phenolic compounds which are concentrated in the outermost portions of the wheat kernel, especially in aleurone layer of bran (Parker et al. 2005, Anson et al. 2008). Importantly, wheat phenolic compounds are not equally soluble or even readily soluble as they are constituent components of bran cell walls and its fibre fraction. Chemically, phenolic compounds can be fractionated as free, soluble conjugated (or esterified) and insoluble bound forms (Krygier et al. 1982, Liyana-Pathirana and Shahidi, 2006, Hung et al. 2009). 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. Kim et al. (2006) also reported high levels of AOA for the bound phenolic fraction of wheat bran compared to free phenolics. The above cited papers clearly indicate that most of the antioxidants present in wheat bran are in bound form, and that it is important to release bound phenolics from cell walls to evaluate the antioxidant capacity of wheat bran.

## 2.5. Definitions

### 2.5.1. Dietary fibre

Dietary fibre is found in many food sources like fruits, cereals, vegetables, etc (Davidson and Mcdonald, 1998). A definition provided by the Codex Commission on Nutrition and Foods for Special Dietary Uses (CCNFSDU) in 2008, states that dietary fibres are carbohydrate polymers consisting of ten or more monomeric subunits that are not hydrolyzed by endogenous enzymes present in the human small intestine (Kendall et al. 2010). Three categories that these polymers can belong to are: 1) natural carbohydrates found in foods, 2) carbohydrates obtained from raw materials by physical, enzymatic or chemical processes, and 3) synthetic carbohydrates proven to have a physiological effect to human health (Kendall et al. 2010). Dietary fibre can be categorized based on how it reacts with water (Davidson and Mcdonald, 1998). Soluble fibre are able to form a dispersion when mixed with water and form a viscous gel in the intestinal tract as opposed to insoluble fibres which cannot (Davidson and Mcdonald, 1998). Soluble fibres include β-glucan, pectins and gums for the most part, whereas insoluble fibres are comprised of compounds such as cellulose, arabinoxylan, and lignin (Davidson and Mcdonald, 1998). A portion of pentosans or arabinoxylans in wheat are soluble, but solubility depends on the wheat fraction, as wheat endosperm pentosans are much more soluble than those in bran. About 30% of total pentosans in wheat endosperm are water-soluble and are constituted mainly of arabinoxylans but also contain some arabinogalactans (Faurot et al. 1995). Wheat bran on the other hand is mostly comprised of insoluble pentosans. Maes and Delcour (2002) reported that only 6% of arabinoxylans in a sample of Netherlands' sourced wheat bran was water

extractable. Similarly, Wang et al. (2006) reported ratios of water-extractable to water-unextractable pentosans of coarse wheat bran and typical millstreams in straight grade flour, i.e. endosperm (M1-M3, B1-B4) of 0.02-0.03 and 0.23-0.48, respectively.

### 2.5.2. Antioxidants

Antioxidants can be classified as substances that are able to significantly delay or inhibit oxidative processes when present at low concentrations (Vaya and Aviram, 2001). Antioxidants in humans (exogenous and endogenous sources) consist of proteins, carbohydrates, phytophenols, minerals, and vitamins (Seal, 2006). Phytochemicals (like FA) are important in maintaining the equilibrium of antioxidants and oxidants in the human body (Adom and Liu, 2002). Having a disparity of these levels can cause oxidative stress leading to oxidative damage (Adom and Liu, 2002). Some diseases (some mentioned below) have been linked to the injury of biomolecules like DNA, proteins, and lipids (Willcox et al. 2004).

## 2.6. Links between Cardiovascular Disease, Soluble Fibre and Antioxidants - Background

Cardiovascular Disease (CVD) is the leading cause of global deaths as reported by the World Health Organization (WHO, 2011a). In 2004, CVD related deaths represented 29% of the world's population or 17.1 million people (WHO 2011a). There is strong epidemiological evidence of increased intake of whole grains related to the

prevention of chronic diseases, such as coronary heart disease in particular (Andreasen et al. 2001).

### 2.6.1. Antioxidants and CVD

Atherosclerosis is a pathophysiological state in which the arteries slowly undergo thickening of intima (innermost coat of organs including blood vessels and arteries) (Matsuura et al. 2008). This condition results in diminished elasticity, narrowed arteries, and decreased blood supply that can lead to angina pectoris, myocardial infarction, and cerebral infarction (Matsuura et al. 2008). The onset of atherosclerosis in animals and humans is widely believed to be the result of low density lipoprotein (LDL) modification through oxidation (Matsuura et al. 2008). LDLs are the main lipid transporters in plasma made up of cholesteryl ester, phospholipid, free cholesterol, triglyceride, and apolipoprotein B-100 components (Matsuura et al. 2008). LDLs are able to build up in the subendothelial space of arteries where they are slightly oxidized by endothelial cells, macrophages, and smooth muscle cells (Diaz et al. 1997). The mildly oxidized LDLs promote the recruitment and conversion of monocytes to macrophages by producing monocyte chemotactic proteins, granulocytes, and macrophage colony stimulating factors (Diaz et al. 1997). Macrophages and monocytes cause further oxidation of LDL, but more importantly the apolipoprotein B-100 constituent becomes more negatively charged (Diaz et al. 1997). This change in LDLs allows them to be recognized by scavenger receptors on macrophages and internalized to form foam cells (Diaz et al. 1997). Foam cells are the identifying feature of fatty

streaks, the first visible lesion of atherosclerosis in animals and humans (Steinberg, 2009). Oxidized LDL as opposed to normal LDL creates cholesterol rich foam cells due to negative feedback regulation not present in macrophage receptors that would minimize its uptake (Diaz et al. 1997). Evidence of oxidized LDL antibodies only reacting with atherosclerotic lesions but not normal arteries supports the oxidation modification hypothesis (Diaz et al. 1997). LDL oxidation is believed to be highly atherogenic which leads to an increase in CVD risk (Seal, 2006).

In light of the above observations, it is believed that antioxidants can play an important role to lower LDL oxidation and therefore reduce onset of CVD. Numerous studies have shown that cereal sources as well as fruits and fruit juices have ample levels of antioxidant capacity to reduce LDL oxidation (Jiang et al. 2007; Ohta et al. 1997; Kaliora et al. 2009; Ignazio et al. 2010; Seeram et al. 2008). Accordingly, the corresponding link to consumption of wheat bran, with its high levels of phenolic antioxidants, is obvious. In this respect, De Moura et al. (2009) concluded that "a whole grain and CVD health claim is supported using a broader concept of whole grain typically used in the scientific literature that includes whole grain foods containing principal components such as bran.

### 2.6.2. Soluble fibre and CVD

Soluble fibre has been attributed to lower risk of CVD as a result of its hypocholesterolemic (cholesterol reduction) effects in the human body (Anderson et al. 2009). The principal mechanism of the cholesterol lowering by soluble fibre is believed

to be related to its ability to increase viscosity of luminal contents, bind bile acids, and excrete them from the body (Salas-Salvado et al. 2006). Soluble fibre raises the viscosity of intestinal contents due to its hydrating characteristic which results in a resistance to bulk diffusion in the gut (Salas-Salvado et al. 2006). This viscous property of soluble fibre coupled with its binding ability of bile, which contains cholesterol, leads to reduced uptake of bile acids at its main site of absorption, i.e. the distal ileum in the small intestine (Salas-Salvado et al. 2006).

Another theory about the hypocholesterolemic property of soluble fibre is its ability to be fermented in the colon and produce short-chain fatty acids that are able to reduce cholesterol synthesis in the liver (Anderson et al. 2009). Cholesterol lowering is important to mitigate CVD because of the involvement of LDL; it has been reported that a 1% decrease in serum LDL cholesterol levels resulted in a 1-2% reduction of coronary heart disease events (Kendall et al. 2010). A review by Anderson et al. (2009) of randomized, controlled clinical trials found reductions in LDL cholesterol of 10.6, 13.0, and 11.1% when subjects consumed divided doses of 9-30, 12-24, and 5 g/d of guar gum (4 trials), pectin (5 trials), and barley β-glucan (9 trials), respectively. In principle, wheat bran with its very low level of soluble fibre should have little to no influence on reducing total cholesterol or LDL. In fact, no reports supporting this link have been published as far as this writer is aware. However, if the solubility of bran fibre could be increased by pre-treatment, the value of bran as a functional food should be increased significantly.

### 2.6.3. Cancer and antioxidants

According to the WHO, cancer is the leading cause of death in the world and has contributed to 13% of global deaths (WHO, 2011b). The predominant forms of cancer are breast, colon, lung, stomach, and liver (WHO, 2011b). Global deaths caused by cancer are expected to climb from 7.6 million in 2008 to over 11 million by 2030 (WHO, 2011b).

A large body of experimental evidence points towards free radicals playing a part in the initiation and promotion of cancer (Willcox et al. 2004). The development of cancer is the result of a single cell modification caused by either inheriting a genetic anomaly or through DNA strand damage caused in part by free radicals (WHO, 2011b; Willcox et al. 2004). It is known that free radicals naturally occur in body fluids and organs as a result of respiration, causing t-mutations from oxidative DNA damage (Collins, 2005). DNA damage caused by reactive oxygen species (i.e. free radicals) could be seen in modifications of pyrimidine, purine, or deoxyribose, including single- or double-stranded DNA breaks, and DNA cross-links (Valko et al. 2007). Carcinogenesis linked to oxidative DNA damage can be the result of either genomic instability, replication errors, the stoppage or stimulation of transcription or signal transduction pathways (Valko et al. 2007). So, antioxidants in the diet are believed to function as preventative agents to reduce the incidence of cancers. While not confirmed scientifically, this property of antioxidants is believed to be the reason why fruits, vegetables and whole grains or fractions such as bran can reduce cancer rates as reported in numerous observational studies (Collins, 2005).

The role of phytic acid, which as already noted is present in very high levels in wheat bran (Table 2), as a potent dietary anti-carcinogen is discussed later in this literature review. However, it deserves mention here as a few studies have noted its antioxidant activity (Graf et al. 1987, Graf and Eaton 1990, Martinez-Tome et al. 2004). That antioxidant activity may be related to the ability of phytic acid to bind divalent cations and reduce their bioavailability. This is the reason why phytic acid has traditionally been considered to be an anti-nutrient (most notably iron deficiency anemia). However, as phytic acid can form unique iron chelates, it suppresses iron-catalyzed oxidative reactions including lipid oxidation (Graf and Eaton, 1990). Many studies have indicated efficacy of phytic acid 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).

### 2.6.4. Diabetes and soluble fibre

In 2008, diabetes prevalence in the United States was estimated at 8% or 23.6 million people, of whom 90% had Type 2 diabetes and of that population 80% were obese (Anderson et al. 2009). The WHO reported that more than 220 million people worldwide have diabetes and that deaths caused by diabetes will double between 2005 and 2030 (WHO, 2011c).

Diabetes is known as a chronic disease resulting from the body not being able to produce enough insulin or not being able to use the insulin it produces to successfully metabolize glucose (WHO, 2011c). In diabetes therapy, one of the major objectives is to normalize fasting and postprandial blood glucose concentrations (Miller, 1994). Glycemic index is a term used to quantify the differences in blood postprandial glucose responses for a food (Kendall et al. 2010). To determine the glycemic index of a food, the area under the blood glucose curve is measured and expressed as a percent of a control (e.g. white bread or glucose drink) (Kendall et al. 2010). Soluble fibre through its property to increase intestinal viscosity is able to weaken convective transfer of glucose and water for absorption in the gut (Nuttall, 1993). Ingestion of soluble fibre lowers peak glucose levels as reflected by the reduced area of blood glucose curves compared to consumption of food with lower levels of non-soluble fibre (Davidson et al. 1998). Soluble fibre has been shown to decrease postprandial insulin response and associated hyperinsulinemia (Davidson et al. 1998). Repeated episodes of hyperinsulinemia are believed to contribute to insulin resistance over time by down regulation of insulin receptors (Davidson et al. 1998).

## 2.7. Dietary fibre in wheat bran

Cereal grains with high sources of soluble fibre consist of oat, barley, and rye (Davidson and Macdonald, 1998). Cereal grains are known to be rich in insoluble fibre with the highest amounts found in wheat and corn (Davidson and Macdonald, 1998). Wheat bran has been reported to contain between 36.5 - 52.4% of total dietary fibre

(Vitaglione et al. 2008). The lower levels cited in this review are most certainly inaccurate, and may reflect varying levels of endosperm (i.e. starch) content in bran arising from milling which is invariably not reported, or inaccuracies in determining major fibre constituents such as arabinoxylan whose content in bran has also been reported with a wide range (Table 2).

The insoluble and soluble fibre levels in wheat bran are 35.0 - 48.4 and 1.5 – 4.0%, respectively (Vitaglione et al. 2008). This difference in fibre composition (Table 2.2) contributes significantly to faecal bulk and increased stool weight, which is essential in having normal laxation (Chen et al. 1998; Olson and Schneeman, 2001). A high-fibre diet usually has a lower fat level and thus contributes to lower incidence of obesity. Wheat bran as a result of its high insoluble fibre content has low digestibility in the upper gastrointestinal tract of humans. *In vitro* studies mimicking the pH and digestive enzymes of the human mouth, stomach, and small intestine found digestibility levels for wheat bran and aleurone-rich wheat bran fractions of 13 and 28 - 40%, respectively (Amrein et al. 2003; Wood et al. 2002). Dietary fibre is fermented by the microflora in the large intestine producing short chain fatty acids including acetate, propionate and butyrate which provides protection against cancer in the colon (Cummings et al. 1987). Wheat bran is reportedly poorly-fermented and thus protection against colon cancer is believed to be brought about by dilution of luminal contents instead of production of butyrate for the colonic mucosa (Monsma et al. 2000). Jenkins et al. (1978) tested wheat bran among other fibres e (41 g per meal) in a human study using healthy subjects for potential use in diabetic treatment. Wheat bran consumption was associated with a flattening of both glucose and insulin response. In a later study

using Type 2 diabetes subjects (Jenkins et al. 2002), high-fibre cereal foods including wheat bran (37 g fibre/day) did not improve conventional markers of glycemic control or risk factors for CVD in type 2 diabetes over 3 months.

Elsewhere, human studies showed that wheat bran intake and lower fat intake contributed to lower incidence of large colorectal adenomas which are precursors to colorectal cancer (DeCosse et al. 1989; MacLennan et al. 1995; Shannon et al. 1996). However Fuchs et al. (1999) did not find any correlation between high dietary fibre intake and reduced risks of colorectal cancer or adenoma. Another study by Michels et al. (2005) encompassing 76,947 women and 47,279 men confirmed no significant relationship between fibre intake and colorectal cancer. There are some limitations noted from the last two studies; fibre intake was estimated for one year only and thus did not represent a lifetime eating habit, misleading labels of "dark bread" at the time and interaction of the fibre and other foods consumed (Trissler, 1999). Intake levels giving the health benefit shown in the studies were 22.5 g of wheat fibre/day (both wheat grain fibre supplement and dietary fibre) (DeCosse et al. 1989), 25 g of wheat bran/day (MacLennan et al. 1995) and >0.72 total cereal servings (hot and cold)/day (Shannon et al. 1996). The recommended fibre intake by the 2005 Dietary Guidelines is 14 g/1,000 kcal for the lowering of cardiovascular disease risks and healthful laxation effects to take place, while average fibre intakes in the U.S. are only 14 g/day and thus the health benefits are not achieved yet (Slavin, 2007). Although there are different types of fibre within wheat bran, those mentioned in this paper are the quantitatively predominant and/or most noteworthy health-wise.

### 2.7.1. Cellulose

Cellulose consists of many glucose units all linked together with ß-1,4 glycosidic bonds to form a linear polysaccharide. It is one of the most concentrated fibres in wheat bran (Table 2.2). Cellulose as an insoluble fibre has been connected with decreasing intestinal transit time, improving faecal bulk, improving stool weight and defecation frequency, and binding and excretion of carcinogens (Gebruers et al. 2008). In an 80 day human study by Wrick et al. (1983) involving 24 subjects, cellulose and coarse bran produced significantly shorter transit times compared to cabbage, fine wheat bran, and basal diets. The bulking effect of cellulose was evident (Wrick et al. 1983) and cellulose significantly increased total stool production by 24.9% compared to a basal diet. In another study, Spiller et al. (1980) examined transit time and stool output with cellulose (14 g/d), pectin (6 g/d), and placebo (sucrose) diets that were fed to 42 human subjects over a three week trial. The level of cellulose from wheat bran that would be consumed by a typical human male adult is 1.7 times (See Table 2.2) that used by Spiller et al. (1980) based on the Adequate Intake (AI) fibre level according to Health Canada's Dietary Reference Intake*s (DRI)* recommended consumption of 38 g of fibre (Health Canada, 2010) (76 g wheat bran) per day. The level used by Spiller et al. (1980) is realistic compared to what is regularly ingested. Cellulose again exhibited significantly shorter transit times and higher fecal output compared to placebo and pectin diets (Spiller et al. 1980). Cameron et al. (1989) reported that cellulose levels in the diet of 5 and 15% (w/w) were able to decrease significantly cell proliferation in crypts of the descending colon of rats after exposure to 1,2-dimethylhydrazine (DMH;

carcinogen) by 24.2 and 28.9%, respectively. Similar 31% reduction of cell proliferation was found (Jacobs and Lupton, 1984) when supplementing rat diets were supplemented with 10% (w/w) cellulose. A study by Sloan et al. (1993) found similar reduction of colon cancer risk using a high fibre cellulose diet (28.7% w/w) significantly reduced DMH induced rat colon neoplasms compared to a high lignin or low fibre diet.

### 2.7.2. β-glucan

β-glucan is considered a soluble fibre and is present in low concentration (~ 0.4%) in wheat bran (Table 2.2) which is s considerably lower than that in barley (~ 3%) and oats (~3%) (Henry, 1985). β-glucan is a linear homopolymer arranged in blocks of consecutive β-(1-4)-linked D-glucose residues separated by single β-(1-3)-linkages. Cereal β-glucan (soluble fibre) has received considerable attention due to its beneficial physiological effects to control CVD and diabetes, such as attenuating blood glucose levels and the ability to reduce serum LDL (Liu, 2007). It has been demonstrated that the ability of cereal β-glucan to attenuate blood glucose and insulin levels is related to β-glucan's viscosity (Cui and Wang, 2009). The mechanism for this has been attributed to structure and molecular weight distribution, as well as the solubility of β-glucan (Cui and Wang, 2009). Theuwissen and Mensink (2007) reported that simultaneous intake of β-glucan and plant sterols effectively lowered the serum LDL-cholesterol level of human subjects who were slightly hypercholesterolemic, which potentially provides a new approach to achieve additional reduction of serum cholesterol. Many animal studies have shown that barley as well as oat β-glucan significantly lowers levels of total

cholesterol, LDL cholesterol, and triglycerides (Kalra and Jood, 2000, Kahlon et al. 1993). In human studies, it has also been demonstrated that barley β-glucan lowered serum cholesterol and attenuated insulin response (Bourdon et al. 1999, Theuwissen et al. 2007). Cereal β-glucans have also demonstrated other health benefits. For example, oat fibre prolonged satiety after meals by decreasing glycaemic index, and alleviated constipation (Mälkki and Virtanen, 2001).

Health claims have been allowed in the United States, United Kingdom, Sweden, and the Netherlands for both oat and barley β-glucans for lowering the risk of coronary heart disease (FDA, 1997 and 2006, Ames and Rhymer, 2008), based on substantial scientific evidence from both animal models and human clinical trials. The United States Food and Drug Administration (FDA) claimed that daily consumption of 3 g of soluble β-glucan from whole-grain barley or certain dry milled barley products would produce the same cholesterol-lowering effect as oat products (lowering plasma total cholesterol by 5–8%). Based on this assessment by the FDA, the amount of wheat bran consumed according to adequate intake (AI) fibre recommendations given by Health Canada's recommended daily intake for an adult male (38 g/d) (Health Canada, 2010) would include only 0.30 g/d (See Table 2.2) of β-glucan, which is much lower than the FDA recommendation. In two-layer flat bread made wheat flour, addition of 20% barley fibre-rich-fractions increased total β-glucans from 0.2 g to 3.0 g, soluble β-glucans from 0.09 g to 1.43 g, and arabinoxylans from 2.4 g to 4.2 g per serving (Izydorczyk et al. 2008). Accordingly, this formulation enhancement made a significant contribution to the recommended daily intake of both soluble and insoluble dietary fiber

(20–35 g per day for the healthy adult population) (American Dietetic Association, 1993).

2.7.3. Arabinoxylan

Arabinoxylans are non-starch polysaccharide polymers which consist of a backbone of β-1,4 linked D-xylopyranosyl residues substituted at O-2 and/or O-3 with α-L-arabinofuranosyl residues. Arabinofuranosyl residues can be substituted at O-5 with FA (Gebruers et al. 2008) by phenolic ester cross-linking (Pomeranz, 1988). Arabinoxylan in wheat exists in both soluble (endosperm = partially water soluble) and insoluble (pericarp = mainly insoluble) fibre forms, with the latter being rich in phenolic acids (Vitaglione et al. 2008; Nystrom et al. 2009). Arabinoxylan (AX)-rich fibre was shown to have similar physiological properties as rapidly fermentable and soluble fibre in the large bowel of rats, while soluble fibre is well-known for its beneficial effects on carbohydrate metabolism (Lu et al. 2000a). Subjects eating breakfast containing AX-rich fiber (6 and 12 g) were found to have improved postprandial glucose and insulin responses compared to 0 g fibre consumption. A possible mechanism was that the solubility of AX and thus its high viscosity likely brought about a reduced rate of gastric emptying and small intestine motility, and these events result in delayed glucose absorption or flattened blood glucose response (Lu et al. 2000b). Similar responses were observed in a study of human participants with impaired glucose tolerance after dietary intervention with addition of 15 g AX-rich fibre (Garcia et al. 2007). Significantly lower postprandial serum glucose and insulin responses were reported (Garcia et al. 2007). In another human study, glycaemic control was found to improve

in people with Type II diabetes with consumption of 15.1 g of AX-rich fibre/day (Lu et al. 2004). The level of AX consumed from wheat bran by a typical human male adult based on the AI fibre level according to Health Canada (2010) would range from 6.76 – 21.28 g/d (See Table 2.2), which is in the range of amounts used in the studies just mentioned.

### 2.7.4. Fructans

Fructans consist of one or more fructosyl-fructose links that make up the majority of the glycosidic bonds. In plants, fructans can range from three to hundreds of fructose units (Roberfroid and Delzenne, 1998; Ritsema and Smeekens, 2003). Fructan can be either linear or branched, and β-(1-2) and β-(6-2) linked in wheat sources (Haska et al. 2008). Fructans (soluble fibre), especially inulin-type fructans (inulin and oligofructose), are considered as functional food ingredients since they affect physiological and biochemical processes in rats and humans, resulting in better health and reduction in the risk of many diseases (Kaur and Gupta, 2002). The Inulin-type fructan term covers oligofructose (degree of polymerization (DP) 2-8, $DP_{av} = 4$), native inulin (DP 2-60, $DP_{av} = 12$), inulin HP (DP 10-60, $DP_{av} = 25$), and Synergy 1 (a commercial product combining oligofructose and inulin HP), a combination of inulin HP and oligofructose (Roberfroid, 2007). Scientific evidence (experimental (E) and/or human (H)) has been found that inulin-type fructans selectively stimulate bifidobacteria growth in the colon (H+E), increase fecal bulking (H+E), improve bioavailability of calcium (H+E), reduce cholesterol (E), lower triglycerides (E), and modulate blood glucose (H+E) (Roberfroid and Delzenne, 1998). Health benefits attributed to inulin-

type fructans include reduced intestinal infections and colon cancer (microflora change), decreased osteoporosis risk (calcium bioavailability effect), reduction of cardiovascular disease risk reduction and obesity (lipid metabolism modification), and improved insulin response. A large number of animal studies and preliminary clinical data show that fructooligosaccharides in the diet increase satiety, reduce energy intake, reduce body weight gain, reduce fat mass development, and reduce serum triglyceride accumulation induced by a high-fat diet (Cani et al. 2005, 2006; Daubioul et al. 2002). Despite the numerous studies that have been done with inulin-type fructans the only definitive (as opposed to promising) is its prebiotic effect on colonic microflora (Roberfroid and Delzenne, 1998). *Bifidobacterium,* bacteria is considered a beneficial species (for health) in contrast to harmful bacteria in the colon, such as *Escherichia coli* and *Clostridium perfringens* (Gibson et al. 1995). A human study by Gibson et al. (1995) demonstrated the prebiotic ability of inulin-type fructans, oligofructose and inulin by implementing a 15 g/d diet of each over 15 days to significantly increase bifidobacteria from 8.8 to 9.5 $\log_{10}$ g/stool and 9.2 to 10.1 $\log_{10}$ g/stool, respectively. The inulin-type fructans were able to significantly modify the fecal microbiota and become the predominant bacteria in the feces (Gibson et al. 1995). The level of fructan consumed from wheat bran by a typical human male adult based on the AI fibre level according to Health Canada (2010) would range from 2.58 – 3.34 g/d (See Table 2.2), which is well below the level used in the Gibson et al. (1995) study.

2.7.5. Lignin

Lignins are non-polysaccharide cell wall substances that are mainly derived from the three monolignols: *p*-coumaril, coniferyl, and synapyl alcohols. Lignin as an insoluble fibre is able to increase bile acid excretion (bile acid binding ability) and fecal bulk. Like cellulose, lignin is also fermented at low levels or not at all by bacteria in the gut due to their complex structure (Story and Kritchevsky, 1976, Blaut, 2002). Also, lignins lower intestinal transit time, which is important to reduce exposure time of hydrophobic carcinogens adsorbed to the fibre (Moore et al. 1998). Lignins also possess *in vitro* antioxidant activity and oxidative DNA damage protection properties (anti-mutagenic) (Fardet, 2008). A study of lignin fed to rat with rye and wheat bran comprising 15% of total diet found that lignin acted as precursors of mammalian lignans which are likely to contribute to reduced risk of breast cancer and coronary heart disease (Begum et al. 2004). Lignin can bind nitrates, carcinogens, bile salts, amino acids and possibly minerals in the gastrointestinal tract and also inhibits microbial growth and enzymatic digestion along with phenolic monomers (Jung and Fahey, 1983). However, Lindner and Moller (1973) found that lignin might not be a cholesterol-lowering agent, as it was found to increase plasma-cholesterol. Lignin did not have any effect upon plasma cholesterol in chickens even though it is present in high amounts in wheat bran in the chicken diet (Weiss and Scott, 1978). A case-control epidemiological study with over 6,107 subjects, examined the connection between lignin intake and colorectal cancer risk (Negri et al. 1998). A 17% relative risk reduction was found when comparing extreme quintiles of lignin consumption (high vs. low): > 2.68 vs. < 1.49 g/d

(Negri et al. 1998). Lignin consumption from wheat bran by a typical human adult male based on the AI fibre level according to Health Canada (2010) would range from 2.66 – 3.95 g/d (See Table 2.2), which is in the range of the colorectal cancer risk reduction described by Negri et al. (1998).

## 2.8. Human digestion (stomach, and small Intestine)

Digestion consists of the physical reduction of a meal into a small particle suspension and the chemical transformation of molecules into a form able to cross the intestinal lining (Barrett, 2006a). Factors influencing digestion include pH, enzymes, and biological detergents (Barrett, 2006a).

### 2.8.1. Stomach

The gastric phase provides the first significant stage of digestion (Dempsey, 2010). Ingested food creates a stimulus for HCl (acid) secretion creating a fasting pH range 1.4 – 2.0 (n=24) and 1.1 – 1.6 (n = 79) in old and young subjects, respectively (Barrett, 2006b; Russell et al. 1993). A mixture known as chyme is formed in the stomach, which is a combination of ingested material and gastric secretions (Wildman and Medeiros, 2000). The acidic conditions of the stomach are able to denature proteins, activate pepsin, liberate nutrients, and at least partly sterilize microbes contained in meals (Wildman and Medeiros, 2000). Pepsin, an endopeptidase is able to catalyze the hydrolysis of proteins in the stomach at an optimum pH of 2.5 and is inactive above a pH of 5 (Dempsey, 2010). Before entry into the small intestine the stomach imparts mixing and propulsion for controlled release of chyme into the

duodenum (Wildman and Medeiros, 2000). Meal composition which includes high fat or high fibre has been shown to slow gastric emptying (Ekmekcioglu, 2002). The postprandial gastric half emptying time of humans consuming a fibre enriched meal as determined by Degen and Phillips (1996) ranged from 153 – 202 min (n = 32). Table 2.3 gives other examples of GI transit times for meals containing bran/fibre.

### 2.8.2. Small intestine

Upon entry into the small intestine, partially digested material from the stomach causes pH to rise, as bicarbonate and water are secreted from the pancreas and Brunner's glands (Wildman and Medeiros, 2000). The SI digested contents possess a pH close to neutral in two human studies a pH of 6.0 (n=10) (Mojaverin, 1996) and 7.3 (n=66) (Pye et al. 1990) was found. Bile, pancreatic and intestinal juices nearly neutralize (pH 6.0-7.0) acidic chyme when food enters the first part of the SI, the duodenum (Barrett et al. 2010). The pancreas plays a major role in digestion as it secretes important proteolytic, lipolytic, amylolytic, and nuclease enzymes (Wildman and Medeiros, 2000). Bile secretions from the liver are composed of bile acids, bile pigments, and other substances dispersed in alkaline solution (Barrett et al. 2010). Bile salts possess an amphiphatic property enabling them to reduce surface tension and aid in the emulsification of fat necessary for its digestion and absorption (Barrett et al. 2010). Human GI transit times of medium to high bran/fibre levels are listed in Table 2.3.

2.8.3. *In vitro* digestion models

*In vitro* models used to study digestion are generally classified into one of two categories, i.e. static or dynamic (Venema et al. 2009). Dynamic models involve the flow of liquid and pH adjustments made over a period of time (Venema et al. 2009). Static models consist of applying a test material to digest solution batch-wise, for a set time at a fixed temperature, and pH (Venema et al. 2009). Digestion models can be further categorized as a single (one GI region simulated i.e. mouth, stomach, SI, or colon) or a multiple step model (two or more regions are simulated) (McClements and Li, 2011). Several important factors contribute to making an *in vitro* digestion model comparable to *in vivo* conditions such as incubation time, pH changes, enzyme composition and activities, and digest volume. Enzyme activity is believed to be the most important factor in *in vitro* digestion models and is affected by concentration, pH, incubation time, activators, inhibitors or buffers, and temperature (Hur et al. 2011). The *in vitro* digestion method used in this thesis research was similar to many other published methods that do not include the large intestine for assessment of digestion products. Since most of the absorption of food nutrients and phytochemicals takes place in the SI, questions of bioaccessibility can be studied based on studies of the upper GI tract (Brandon et al. 2006; Carolien et al. 2005). Whereas bioavailability describes the fraction of a food (or drug) that enters systemic circulation and tissues and requires access to biological fluids for quantification, bioacessibility as measured by changes in the solubility of food components, reflects the potential of a substance to be absorbed. Clearly bioaccessibility is more easily studied. Researchers use *in vitro* digestion models of the upper intestinal tract (mouth, stomach and SI) to remove digestible

components and to detect changes in non-digestible components (Aura, 2005), in effect to measure bioaccessibility.

The mouth in digestion consists of chewing food at forces of 300-1000 N in the presence of saliva, which contains most notably α-amylase (starch digestion) and mucin (lubrication and surface protection) (Aura, 2005). The mouth (saliva secretion) step in the *in vitro* digestion process has been included in some studies (Lan-Pidhainy et al. 2007; Wood et al. 2002; Shim et al. 2010; Beer et al. 1997; Lebet et al. 1998), but in others has been omitted leaving a gastric-small intestine model (Minekus et al. 1995; Gil-Izquierdo et al. 2002; Miller et al. 1981; Tarko et al. 2009; Tsai et al. 2008). This can be justified in part on the minor effect that α-amylase has in the mouth due to the very limited time for starch digestion compared to what occurs in the SI by α-amylase secreted from the pancreas (Tavakkolizadeh et al. 2010). Salivary α-amylase is not essential for the normal digestion of carbohydrates (i.e. more important for infants and patients with pancreatic insufficiency) since pancreatic α-amylase is produced in substantially higher amounts than what is s required (Barret, 2006c). Also, in the case of bran, simulating the chewing of food (mastication) is also not essential in *in vitro* digestion as the hydrated bran is already homogenous and has small particle size. Furthermore, the amount of time a food or beverage spends in the mouth is very short (5-60 s) and is much shorter for liquids (McClements and Li, 2011). Samples that would require time for mastication for swallowing could be homogenized before being placed in the stomach section of an *in vitro* model, like the homogenized broccoli analyzed by Vallejo et al. (2004). Samples that require little to no chewing to allow for

suitable swallowing would be passed from the mouth to the stomach in little to no time such as fine particle size wheat bran as used in this thesis research.

A frequently employed static model constructed by Miller et al. (1981) uses a two-step digestion process involving the stomach and SI. The stomach section consisted of lowering pH to 2 using HCl and simulating the SI using a dialysis bag for both neutralization and absorption. The dialysate would model serum available compounds while the retentate would model color available food constituents. In contrast to Miller's dialysis method for bioavailability (actually bioacessibility) assessment, Toor et al. (2009) centrifuged digest solutions and the soluble supernatant was used to test for bioaccessibility. Miller et al. (1981) chose a gastric transit time of 2 hr based on convenience and results showed 50 – 180 min pepsin incubations had the same iron release. Miller et al. (1981) also chose a time of 2.5 hr for SI transit due the analytical method's (bathophenanthroline) ability to accurately measure dialysate iron concentrations (Miller et al. 1981). Also, 2.5 hr accommodated time required for a rise in pH after the gastric phase through dialysis that would be suitable for pancreatin activity (Miller et al. 1981). A pH of 2 in the gastric phase was used based on frequently reported values of pH 2 in the pyloric region of the human stomach (Miller et al. 1981). A pH range of 3.8 – 7.8 reported for aspirated samples from the proximal and distal region of the duodenum was the foundation for the SI pH of 5 chosen in the Miller et al. (1981) model.

A dynamic computer controlled GI model called TIM (TNO gastrointestinal model) system (stomach and SI) used human GI transit times for yogurt as the basis for implementing a gastric and ileal half delivery time of 70 and 160 min, respectively

(Minekus et al. 1995). Gastric pH levels used were based on *in vivo* results of milk ingestion by human subjects, which resulted in a drop in pH from 4.8 to 1.7 over a 2 hr period (Minekus et al. 1995). The pH of the human duodenum was 6.5, resulting in the use of a similar pH for the SI model (Minekus et al. 1995). The TIM model in an attempt to accurately mimic humans and monogastric animals also includes squeezing, peristaltic movements, and absorption of nutrients and water in the small intestine (Yoo and Chen, 2006). In order to control the electrolyte, enzyme, and bile concentrations along with body temperature and pH, secretions of gastric, pancreatic, and bile are infused into the TIM model (Yoo and Chen, 2006). The TIM model was recently used to determine the bioaccessibility of FA from wheat bran and aleurone which was reported to be very low (< 1%), as well as the FA contribution to antioxidant activity of bioaccessible compounds (< 5%) (Anson et al. 2009a, 2010).

Table 2.3. Human gastrointestinal transit times for bran of fibre

| Test meal | Subjects | Bran or Fibre (g) | Average gastric emptying time (min) | Average small intestine transit time (min) |
|---|---|---|---|---|
| Tc99m-labelled rice pudding meal[1] | 12 | 15 (bran) | 50% emptying<br>Coarse bran = 85<br>Placebo = 105 | 50% emptying<br>Coarse Bran = 250<br>Placebo = 375 |
| Tc99m-labelled rice pudding meal[2] | 13 | 15 (bran) | 50% emptying<br>Coarse bran = 110<br>Control = 88 | 50% emptying<br>Coarse bran = 227<br>Control = 322 |
| Tc99m-labelled rice pudding meal[3] | 12 | 15 (bran) | 50% emptying<br>Coarse bran = 121<br>Fine bran = 104<br>Control = 99 | 50% emptying<br>Coarse bran = 333<br>Fine bran = 334<br>Control = 368 |
| Omelet, eggs, and white bread[4] | 14 | 7.4 (fibre) | 50% emptying<br>*Solid*<br>Fibre = 88.4<br>Placebo = 88.7<br>*Liquid*<br>Fibre = 67.5<br>Placebo = 67.0 | N/A |
| Pasta and hamburgers[5] | 8 | 20 (fibre) | 100% emptying<br>20 g Fibre = 231.7<br>4 g Fibre = 186.0 | N/A |
| Scrambled eggs and whole wheat bread[6] | 32 | 15 (fibre) | 50% emptying<br>Fibre = 153 – 202 | 10% emptying<br>Fibre = 181 – 210 |

Data sources for Table 2.3:

1. Hebden et al. (2002)
2. McIntyre et al. (1997)
3. Vincent et al. (1995)
4. Rigaud et al. (1998)
5. Benini et al. (1995)
6. Degen and Philips (1996)

Compared to complex dynamic models of digestion, static models are relatively simple but have varied in scope. Static models of the stomach have ranged from simple models using a highly acidic pH, use of pepsin enzyme alone, and simple mechanical stirring, to complex models that also include simulated gastric fluids containing buffers, salts, organic molecules, biopolymers, and phospholipids (McClements and Li, 2011; Shim et al. 2010; Oomen et al. 2003; Miller et al. 1981). Simulated small intestine models span from use of pancreatin enzyme and bile salt mixtures incubated at a neutral pH to more sophisticated models further adding co-enzymes, buffers, salts, proteins, phospholipids, and small organic molecules (McClements and Li, 2011; Shim et al. 2010; Oomen et al. 2003; Miller et al. 1981). Variability in outcomes of the more popular static models arises from variations in enzyme levels, pH, and incubation times. The advantages of the TIM model compared to models of static *in vitro* digestion include the removal of products of digestion at each stage of digestion, more realistic mixing and physical transport of the digest (Minekus et al. 1995). Despite these apparent advantages, the TIM system has its share of disadvantages in predicting *in vivo* condition (Yoo and Chen, 2006). Such shortcomings include the stirred reactors inability to reproduce shear force and fluid mechanics, gastric and GI secretions not being controlled by feedback mechanisms of the central nervous system and hormonal specific hormone releases, and the natural *in vivo* absorption only being represented by simple diffusion (Yoo and Chen, 2006). A disadvantage of all pre-set *in vitro* digestion models is the presumed low accuracy that will likely arise when compared to *in vivo* results, if factors caused by the food or beverage are not considered i.e. different foods likely require different settings for the key factors of enzyme levels, pH and incubation

time (Lentz, 2008). Presumably if correct choices are made for a particular food, a static digestion model should generate accurate results of *in vivo* digestion with respect to bioaccessibility measures. So, despite the large differences in static and dynamic models the most important component in choosing an *in vitro* model is its correlation with specific food or beverage *in vivo* results.

Tables 2.4 and 2.5 list *in vitro* parameters of enzyme activities, sample weights, and sample volumes used in a range of studies in compared to parameters chosen for this thesis research. The volume used for the simulated digestion (stomach = 900 ml and SI = 1000 ml) in this thesis research is based on published reports of *in vivo science* that after a typical meal approximately 1000 ml and 833 ml of fluid is secreted by the stomach and SI (Fordtran and Locklear, 1966; Doherty and Way, 2010). The GI incubation times selected for the thesis study (stomach = 2.5 h and SI = 5 hr) were chosen based on transit times listed in Table 2.3. *In vitro* models (Tables 2.4 and 2.5) using cereal samples that highly correlated with *in vivo* digestibility in pigs, helped determine the activity units of pepsin (Regmi et al. 2009) and pancreatin (Boisen and Fernandez, 1997; Regmi et al. 2009) used in this thesis research. Pigs were used as the benchmark for enzyme concentrations because of their physiological and immunological similarities with human beings that makes them important for biomedical research (Helm et al. 2002).

Table 2.4. Pepsin activity in relation to sample weight and volume

| Sample type | Sample wt (g) | Vol. (ml) | Specified Pepsin activity (units/mg) | Pepsin added (mg) | Pepsin activity added (units) | Pepsin activity (units/ 900 ml) |
|---|---|---|---|---|---|---|
| Maize-soy[1] | 1 | 3 | - | - | 3,000 | - |
| Strawberry[2] | not given | not given | - | - | 31,500 | - |
| Brocolli[3] | not given | 40 | - | - | 31,500 | 708,750 |
| Various meals[4] | 10 | 90 | 806.3 | 235.2 | 189,641 | 1,896,410 |
| Milk or Soy formula[5] | 10 | 100 | - | - | - | 2,580,160 |
| Oat muffins[6] | 5 | 100 | 3,000est | 0.312 | 936 | 8,424 |
| Wheat[7] | 1 | 37 | 806.3est | 10 | 8,060 | 196,054 |
| Whey protein gels[8] | 0.3 | 150 | 3,000est | 35.4 | 106,200 | 637,200 |
| Raspberry concentrate syrup[9] | 2.5est | 20 | 3,850est | 1.64 | 6,300 | 283,500 |
| Feed[10] | 0.5 | 7 | 3000 | 7 | 21,000 | 385,714 |
| Wheat bran[11] | 100 | 900 | 806.3 | 117.2 | 94,498 | 94,498 |

*Unless stated otherwise the *in vitro* digestion method used was a static model

Data sources for Table 2.4:

1. Zyla et al. (1995)
2. Gil-Izquierdo et al. (2002)
3. Vallejo et al. (2004)
4. Luten et al. (1996)
5. Shen et al. (1994)
6. Beer and Wood (1997)
7. Regmi et al. (2009)
8. Tedeschi et al. (2009)
9. McDougall et al. (2005)
10. Meunier et al. (2008)
11. Golom (MSc 2011)

Table 2.5. Pancreatin activity in relation to sample weight and volume

| Sample type | Sample weight (g) | Vol. (ml) | Pancreatin activity | Pancreatin added (mg) | Wt of Pancreatin (mg/L) | Wt of Pancreatin (mg/ L) Standardized to 4 x USP** |
|---|---|---|---|---|---|---|
| Maize-soy[1] | 0.5 | 2.65 | 8 x USP | 2.405 | 908 | 1,815 |
| Soil and dough[2] | 200 | 600 | 1 x USP | 210 | 350 | 88 |
| Various meals[3] | 10 | 115 | 4 x USP | 100 | 870 | 1,000 |
| Legumes[4] | 2.5 | 38.5 | 4 x USP | 10 | 260 | 260 |
| Oat muffins[5] | 5 | 102.1 | 8 x USP | 0.625 | 6 | 12 |
| Wheat[6] | 1 | 55 | 4 x USP | 150 | 2,727 | 2,727 |
| Whey protein gels[7] | 0.3 | 400 | 1 x USP | 7,000 | 17,500 | 70,000 |
| Feed[8] | 0.5 | 52.5 | 4 x USP | 100 | 1,905 | 1,905 |
| Wheat bran[9] | 100 | 1,000 | 4 x USP | 1,900 | 1,900 | 1,900 |

* Unless stated otherwise the *in vitro* digestion method used was a static model
** 1xUSP per mg = no less than 25 units of amylase activity, 25 units of protease activity, and 2 units of lipase activity (Murray, 2000).

Data sources for Table 2.5:

1. Zyla et al. (1995)
2. Rodriguez and Basta (1999)
3. Luten et al. (1996)
4. Akilhoglu and Karakaya (2009)
5. Beer and Wood (1997)
6. Regmi et al. (2009)
7. Tedeschi et al. (2009)
8. Boisen and Fernandez (1997)
9. Golom (MSc 2011)

## 2.9. Bioavailability

The ability of a bioactive compound to impart beneficial health effects depends on its bioavailability in the human body. The term bioavailability is complex. It refers to the combination of bioacessibility (availability for absorption), actual absorption along the GI tract, tissue distribution, and bioactivity at a specific site (Anson et al. 2009a). Assessment of bioavailability can be done by evaluating time to achieve maximum concentration of a compound in plasma (plasma peak time) and area under the curve (AUC) (of the plasma concentration curve profile) (Kopacek, 2007). The plasma peak time for a compound is the most commonly reported measure of absorption rate; higher peak time values equate to slower absorption and vice-versa (Kopacek, 2007). The AUC method is the most reliable measure of bioavailability. This value is directly proportional to the amount of unchanged compounds in the systemic circulation (Kopacek, 2007). The percent of intact ingested bioactives in the urinary excretion is also used as a measure of bioavailability, due to the possible exposure of tissues to the substance (Scalbert and Williamson, 2000). Bioactive recovery level from ileostomy-tested subject's fecal excretion has also been used to determine absorption levels of compounds (Ross et al. 2003a). Bioavailability of FA in past research has been determined using in situ perfusion of small intestine segment, rat stomach perfusion, animal or human *in vivo* models, and cultured Caco-2 cells of the small intestine and colon epithelium (Poquet et al. 2008). This thesis

research has focused on the first factor limiting bioavailability of wheat bran components, i.e. bioaccessibility.

### 2.9.1. Bioactivity and bioavailability of wheat bran phytochemicals

Phytochemicals ("phyto" = plant) are defined as bioactive non-nutritive compounds found in vegetables, fruits, and grains that have been coupled with risk reduction of chronic diseases (Liu, 2004). The bioavailability of phytochemicals is just as important as their bioactivity, because no health benefits will result if there is not enough bioactive compounds present at the site of action. As mentioned above, the majority of bioactives in wheat are found in the bran. Phytochemicals present in wheat bran are discussed below.

### 2.9.2. Phenolic compounds

Recent evidence for possible favourable health effects from consumption of phenolic compounds in food has pointed towards the risk reduction of cardiovascular diseases, cancers, and osteoporosis (Scalbert et al. 2005), with results supported by epidemiological, *in vitro*, and some animal studies (Halliwell et al. 2005). Phenolic compounds are mainly believed to be beneficial as a result of their antioxidant abilities.

The main classes of phenolic compounds are phenolic acids and flavonoids (Karakaya, 2004). The absorption of phenolic compounds in the GI

tract varies based on their wide range of chemical structures. Most polyphenols are in glycosides, polymers, and esters that are not absorbable in their native form (Manach et al. 2004). Absorption of phenolic compounds in the GI tract depends on esterification (sugars, polymers, etc.) and molecular weight of compounds (e.g. proanthocyanins), coupled with the presence of certain (e.g. esterases) enzymes able to create more absorbable forms (Scalbert and Williamson, 2000). After absorption, phenolic compounds are believed to undergo glucuronidation in the intestinal mucosa, then sulphation in the liver, and finally methylation in the liver and kidney (Azuma et al. 2000). This process increases the hydrophilicity to assist biliary and urinary excretion as seen for other xenobiotics (Manach et al. 2004). Conjugation of phenolic compounds are thought to affect biological efficacy, but up to now little is known of the bioactivity of metabolites in blood and tissues due to a shortage of metabolite commercial standards and imprecise identification of metabolites (Manach et al. 2004). Some studies have shown a decrease in biological properties (e.g. antioxidation) with conjugation compared to *in vitro* studies, but some retention of properties has been reported (Manach et al. 2004). Clearly, more studies are required to fully understand the metabolism of functional compounds. Phenolic compounds due to their wide variation in structure have been shown to have large variations (0.3 - 26%) in bioavailability (Fardet et al. 2008).

2.9.2.1. Phenolic acids (including ferulic acid)

Phenolic acids in wheat bran can be subdivided into two major groups, hydroxylbenzoic acid and hydroxycinnamic acid. Hydroxybenzoic acid derivatives such as *p*-hydroxybenzoic, protocatechuic, vanillin, syringic and gallic acid are typically components of complex structures like lignins and tannins. Hydroxybenzoic acids can also be found as derivatives of sugars and organic acids in plants. Ferulic, *p*-coumaric, caffeic, and synaptic acids are hydroxycinnamic acids and are found mostly in the bound form typically ester-linked to cell wall structural components such as pentosans, cellulose, and lignins (Liu, 2007).

Phenolic acids are believed to have anti-carcinogenic, anti-inflammatory, and antioxidant properties (e.g. can reduce LDL oxidation) (Kylli et al. 2008). The type of antioxidant activities possessed by phenolic acids include radical scavenging, metal ion chelation, and inhibition of prooxidant enzymes (Kylli et al. 2008). An *in vitro* study of the antioxidant activity of FA (main wheat phenolic acid) on liposome oxidation found an over 90% inhibition at a concentration of 10 μM (Bondia-Pons et al. 2009). Another *in vitro* study showed human LDL oxidation inhibition by FA of 55.7% at 10 μM and 24% at 5 μM (Meyer et al. 1998). FA's effectiveness was seen in terms of significant protection of erythrocytes from hemolysis by free or AX-bound FA diets in rat plasma (Rondini et al. 2004). Conversely an *ex vivo* study showed no human LDL oxidation protection by FA after rye bran intake (250 g/d x 6 weeks), even though rye bran significantly increased FA urinary excretions (Harder et al.

2004). FA was able to significantly reduce colonic carcinomas in F344 rats ingesting 250 mg/kg (23% reduction) and 500 mg/kg (27% reduction) of FA with azoxymethane (15 g/kg) (potent carcinogen) compared to azoxymethane alone (59%) (Kawabata et al. 2000). This level of FA is 100 to 200 times (See Table 2.2) the FA that a typical human male adult (wt 81 kg) would ingest from wheat bran based on consumption of 38 g of bran fibre (76 g wheat bran) per day (Health Canada, 2010). Clearly the level of FA used in Kawabata et al. (2000) is not realistic. FA has been extensively studied for its *in vitro* antioxidant effects and has shown to be effective. *In vivo* studies of phenolic acids from cereals like wheat are absent (Fardet et al. 2008). Cereals as significant sources of FA and other phenolic acids require *in vivo* studies to determine if high enough levels of these bioactives are present to provide a health benefit or are too low to be significant as seen in one FA *ex vivo* study (Harder et al. 2004).

Phenolic acids most commonly found in non-cereal grain foods are caffeic acid and to lesser degree FA (Scalbert and Williamson, 2000). Olthof et al. (2001) found absorption values of 95% and 33%, respectively for caffeic acid and chlorogenic acid (quinic acid ester of caffeic acid) in the upper intestines of human ileostomy subjects. Urinary excretion levels after 24 h were 11% and < 1% of initially consumed levels for caffeic acid and chlorogenic acid, respectively. In a rat model study involving sampling of jejunum and ileum digests, caffeic acid and chlorogenic acid (lowest) had absorption levels much lower than those of FA and *p*-coumaric acid (Spencer et al. 1999). Another rat study indicated low chlorogenic acid metabolite (FA and caffeic acid

conjugates) levels compared to caffeic acid metabolites with maximum plasma concentrations after consumption of 0.12 to 0.34 μM in 0.5-1 hr and 1.2 to 26 μM in 2h, respectively (Azuma et al. 2000). Six hours after chlorogenic acid consumption, 98-99% remained unchanged in the small intestine. This implies low chlorogenic acid bioavailability in the small intestine due to low esterase activity (Azuma et al. 2000). It is believed that colonic microbial esterases present in rats and humans are only able to specifically hydrolyze chlorogenic acid (Andreasen et al. 2001a), which could allow for the colonic absorption of caffeic acid. The differing absorption and plasma levels of caffeic and chlorogenic acids seem to result from the ester linkage which, in combination with other structural features of phenolic compounds, is very important in terms of bioavailability.

2.9.2.2. Ferulic acid bioavailability

By consuming 20 g of wheat bran it yields approximately 100 mg of ferulate, 20 mg of diferulate, 3 mg of *p*-coumaric, and 2 mg of sinapic acid (Kern et al. 2003). The absorption of FA in its ester-linked form (polymers or sugars) is not possible, but esterases present in the stomach, small intestine, and colon are able to release FA and di-FA (dimer) for absorption (Kern et al. 2003; Zhao et al. 2004). Andreasen et al. (2001a) demonstrated that the esterase activity in the SI of humans is able to release methyl FA substrate at sizeable speeds (duodenum = 460 ± 150 nmol $g^{-1}$ $h^{-1}$(n = 3), jejunum = 670 ± 290 nmol $g^{-1}$ $h^{-1}$ (n = 3), and ileum = 237 nmol $g^{-1}$ $h^{-1}$ (n = 1)) and that the large intestine

(3200 ± 1800 nmol $g^{-1}$ $h^{-1}$, (n = 4)) (human fecal extract) had release rates 10x that of the SI. In the same study a 25% release of the FA in wheat bran was observed when using human fecal extract esterase (Andreasen et al. 2001a). A more recent study by Poquet et al. (2008) using an *in vitro* colon epithelium model consisting of co-cultured Caco-2 mucus producing HT29-MTX cells, revealed that free FA and to a lesser degree di-FA could be efficiently transported. More research is required regarding FA absorption in the large intestine.

About 0.5 - 5.0% of the total FA (mainly the soluble free fraction) in whole grains is absorbed in the small intestine (Fardet, 2010). FA is also absorbed in the stomach but only in its free form (Zhao et al. 2004). DiFA in bran has also been shown to be absorbed across the gastrointestinal barrier (Andreasen et al. 2001b). FA absorption sites in the gut differ due to the location of esterases. In a rat model study and diets supplemented with FA, Zhao et al. (2003a) found that free FA was absorbed nearly completely before arriving at the cecum. Forty percent of 5-0-feruloyl-$_L$-arabinfuranose (a sugar ester) was absorbed in the rat foregut, while 57% disappeared within the cecum. The FA moiety of arabinoxylans was freed and absorbed at a level of 67% in the hindgut (Zhao et al. 2003a). In humans, cereal FA is believed to be mostly absorbed in the small intestine, with minor portion in the large intestine (Manach et al. 2005). Differences in FA absorption reported by Zhao et al. (2003a) in the high hindgut in contrast to FA absorption in the small intestine (Manach et al. 2005) can likely be explained by the (complex) matrix of the

source of FA (corn bran) interfering with enzymatic breakdown of ester-linked FA as well as differences in digestion between humans and rats.

FA was excreted in urine at levels of 43.4% of the ingested FA consumed (dissolved in water) in rats after 4.5 hr (Rondini et al. 2002). Human urine excretions of total free FA and feruloyl glucuronides reached levels of 11-25% of the FA ingested 24 hr after consuming tomatoes (Bourne and Rice-Evans, 1998). Esterified FA in bran had lower bioavailability than that seen in other esterified FA bioavailability studies. Urinary excretions of human subjects consuming wheat bran, recovered levels of 3.13% FA of the ingested FA dose (Kern et al. 2003). Bioavailability determined by urinary excretions of intact FA in white flour was 53.6% higher than that found in wheat bran (15.3% bran) (Adam et al. 2002). The higher solubility of white flour compared to wheat bran for enzymatic break down is able to explain the dramatic difference of bioavailability (Adam et al. 2002). Results from using 5% refined corn bran diets fed to rats revealed even lower levels of urinary excretions of ingested FA with levels of 0.4-0.5% (Zhao et al. 2005). Digestion barriers caused by the cereal bran matrix including FA esterification are the likely causes of the difference in bioavailability seen between the FA diets.

As mentioned previously, phenolic compounds undergo conjugation during absorption which increases its hydrophilicity (Manach et al. 2004). Studies on FA metabolites have found the free acid form, compared to conjugates, to be a minor portion in the plasma and urine levels analyzed. Zhao et al. (2003b) determined the main FA compound in rat plasma to be FA-sulfo-glucuronide at levels of 60-70% of total FA after consuming diets comprising

free and sugar ester FA. The second highest FA compound recovered at 15 min (peak concentration time) in plasma was free FA followed by FA-glucuronide (Zhao et al. 2003b). A rat-based study of urinary excretion of FA after consumption of diets supplemented with free FA also showed that the highest levels of metabolized FA was the suflated form as sulfoconjugates (sufated and sulfo-glucuronidated FA); levels were 84%, 4.5 hr after the meal (Rondini et al. 2002). The second and third highest forms of metabolized FA were free and glucuronidated FA, respectively (Rondini et al. 2002). The antioxidant ability of only the FA-glucuronide metabolite has been tested. For protection against *in vitro* LDL oxidation, free FA was comparable to its glucuronidated form (Zhao et al. 2003b). The need for FA sulfoconjugates to be analyzed for their *in vitro* antioxidant protection is crucial as it is the dominant FA form in a position (i.e. in plasma) to reduce LDL oxidative damage.

2.9.2.3. Flavonoids

The most abundant polyphenols in the human diet in general are flavonoids as they are very abundant in fruits and vegetables. In whole wheat, flavonoid levels are within the range of concentration of FA, but in bran, flavonoid concentrations are much lower than that for FA (Table 2.2). There are seven flavonoid classes in general which can be differentiated by the degree of oxidation of the oxygen heterocycle: flavones, flavonols, isoflavones, anthocyanins, flavanols, proanthocyanidins and flavanones (Scalbert and Williamson, 2000). Flavonoids are strong antioxidants that have been able to

scavenge reactive nitrogen, oxygen, and chlorine species *in vitro* (Halliwell et al. 2005). They also can act as metal chelators to lower oxidative activity of metal ions (Halliwell et al. 2005). Two epidemiological studies on the effect of catechin consumption in the Netherlands (NL) and United States (US) found substantial reductions of coronary heart disease (CHD) risk (Arts et al. 2001a; 2001b). US (32, 857 females) and NL (806 males) studies compared catechin consumption levels (high vs. low) of 124.0 vs. 25.3 mg/d and 74.8 vs. 3.6 mg/d, respectively (Arts et al. 2001a; 2001b). Relative risk of CHD was reduced by 15% (high risk group) and 51% for US and NL studies, respectively (Arts et al. 2001a; 2001b). As noted above, wheat and other cereal grains possess relatively low amounts of flavonoids relative to fruits and vegetables (Alvarez et al. 2006). Epidemiological evidence (Alvarez et al. 2006) and significant increases in antioxidant abilities found *in vivo* for humans (Leenen et al. 2000), strongly indicates that flavanols (catechins) are important parts of our diet in preventing degenerative diseases arising from oxidative stress occurring in the body. The level of flavonoid consumed from wheat bran by a typical human male adult based on the AI fibre level according to Health Canada (2010) would range from 11.32 – 43.32 mg/d (Table 2.2). This is below levels reported in the Arts et al. (2001b) for CHD risk reduction, but would give a 14 to 17% CHD risk reduction based on the Arts et al. (2001a) study. The conflicting results between the two epidemiological flavonoid studies indicate that more research is needed to determine the amount needed to produce proposed health effects.

Dietary consumption of phenolic compounds consists of 33% phenolics acids and the rest flavonoids, with the main classes being flavanols and anthocyanins and their oxidation products (Scalbert and Williamson, 2000). Anthocyanin glycosides from blueberry extracts were analyzed using Caco-2 cells and revealed average low absorption efficiency levels of 3-4%, with the lowest absorbed being delphinidin glucoside (< 1%) (Yi et al. 2006). The glucose sugar moiety of cyanindin and peonidin anthocyanin glycosides showed significantly higher bioavailability than galactose (Yi et al. 2006). Anthocyanins appear to have a relatively lower bioavailability than other flavonoids, but structural modifications (i.e. glucose attachment) play a factor. Flavanols (catechins) unlike other flavonoids in foods are not glycosylated (Manach et al. 2004). Consuming 2 g of solid green tea (0.32 g/g catechin) and black tea (0.07 g/g catechin) each both significantly increased the catechin human plasma levels by 1.8 μM and 0.34 μM after 90 min, respectively (Leenen et al. 2000). Antioxidant plasma levels determined by the ferric reducing ability of plasma (FRAP) assay after consuming green tea and black tea significant increased by 3% and 2%, respectively (Leenen et al. 2000). The boost in antioxidant ability by flavanols signifies its good efficiency (from 2 g of tea) to affect human bioactivities.

2.9.2.4. Carotenoids

There are two classes of carotenoids, carotene and xanthophylls. Carotenes (α and β carotene, lycopene) consist of only hydrogen and carbon

atoms, while xanthophylls (β-cryptoxanthin, lutein, zeaxanthin) are made up of oxygenated hydrocarbon derivatives that contain at least one oxygen function (hydroxy, keto, epoxy, methoxy or carboxylic acid groups) (Rodriguez-Bernaldo de Quiros and Costa, 2006). Many prospective epidemiological studies on β-carotene (11 out of 15) showed an inverse link of β-carotene plasma levels and/or intake levels and lung cancer risk (Johnson, 2002). Two large long-term randomized intervention trials found β-carotene at high doses (20-30 mg/d) may have a harmful effect on smokers and asbestos workers (high lung cancer risk groups), but no effect on the common well-nourished population (Johnson, 2002). A more recent double-blind randomized study with a long term follow up found no link between β-carotene and lung cancer risk (Kamangar et al. 2006). The inconsistent results of epidemiological studies on β-carotenes illustrate the need for further research with emphasis on clinical trials focusing on lung cancer risk reduction. Some of lutein's reported biological actions relate to *in vitro* antioxidant activity, monocyte-LDL oxidation inhibition, and macular protection (age-related macular degeneration (AMD) (Granado et al. 2003). Dietary intake of carotenoids in human studies was able to increase its concentration in the retina (lutein and zeaxanthin the only carotenoids in retina and lens), which could protect against eye diseases (Johnson, 2002). Consuming 15 mg of lutein x 3/wk for 2 yrs significantly increased visual acuity of human cataract subjects and vision improved (clearer and more recognition) in AMD subjects (Olmedilla et al. 2001). Another human study also found that increasing intake of lutein and zeaxanthin reduced the risk of advanced AMD by 57% for the highest (19.25 mg/d) versus lowest

(3.15 mg/d) quintile of consumption (Seddon et al. 1994). However, contrary results have been reported in a case-controlled eye study, in which no connection was found between serum lutein or zeaxanthin and AMD protection (Seddon et al. 1994).

Lutein consumed from wheat bran by a typical human male adult based on the AI fibre level according to Health Canada (2010) would be 0.038 – 0.14 mg (Table 2.2), which is well below the amount that could improve vision in AMD subjects as reported by Olmedilla et al. (2001). The highest level of lutein and zeaxanthin consumed from wheat bran based on AI fibre recommendations would be 0.30 mg/d, which is well below the 57% AMD risk reducing 19.25 mg/d quintile and even under the lowest quintile of 3.15 mg/d as reported by Seddon et al. (1994).

Important factors that can affect the bioavailability of foods containing carotenoids is the level of food matrix disruption, as well as other food components (e.g. dietary fat and fibre) (Yonekura and Nagao, 2007). Food processing (e.g. heating) can cause disruption of cell walls in foods and organelles which releases carotenoids (Yonekura and Nagao, 2007). Carotenoids are then available for absorption in the small intestine, and largely the duodenum (Yeum and Russell, 2002). Livny et al. (2003) revealed through mass-balance calculations of 15 mg β-carotene meals, that carotenoid absorption in raw carrots was 41.4% compared to 65.1% from cooked (pureed) carrots ($P = 0.048$). Roodenburg et al. (2000) showed that increasing dietary fat content of 36 g vs. 3 g resulted in heighten lutein (esterified mainly to palimitic

acid) plasma levels in contrast to α-carotene or β-carotene. These results indicate that lutein esters due to their higher lipophilicity not seen in regular free lutein (Roodenburg et al. 2000) is more affected by high fat contents for emulsification needed for absorption than α-carotene and β-carotene (also lipophilic).

Source of fat was shown to not affect the micellarizaton of α-carotene and β-carotene but only that of lutein, with fats ranked in effect as follows: vegetable oil > beef > ham > chicken (Garrett et al. 1999). The effect of dietary fibre was studied by Riedl et al. (1999) using standard meals with no fibre (control) or one of five different fibres, i.e. pectin (70% esterified), alginate, cellulose, guar, or wheat bran. Lutein and lycopene human plasma AUC (after 24 h) was significantly reduced by 40-74% for all fibres, while β-carotene was significantly reduced by 33-43% by the water-soluble fibres (alginate, pectin, and guar) (Riedl et al. 1999). The significant reduction of β-carotene's levels in plasma by water-soluble fibres compared to lutein and lycopene may be explained by an increase in viscosity possibly coupled with β-carotene structural differences causing lowered intestinal absorption. More work is required to fully understand this mechanism.

Micellarization of meat and vegetable carotenoid intake was highest in lutein with 25-40% followed by α-carotene and β-carotene with 12-18%, while relatively higher lutein levels were also found in Caco-2 cell micellar uptake levels after 6 hr (Garrett et al. 1999). Lutein-rich foods have been able to significantly increase serum and tissue concentrations of lutein and zeaxanthin (Granado et al. 2003). The higher micelle formation of lutein in aqueous

fractions could be accounted for by their less lipophilic nature allowing for easier solubilisation required for carotenoid absorption. Most studies have shown carotenoid absorption is limited to 20-30 mg due to micellar incorporation, intracellular translocation, chylomicron incorporation and secretion abilities in the digestive tract (Stahl et al. 2002).

2.9.2.5. Lignans

Lignans are polyphenolic compounds found in plants, formed by oxidative coupling of two ρ-propylphenol molecules. Lignans derivatives, enterodiol and enterolactone (health factors), are converted from ingested plant lignan (secoisolariciresinol, matairesinol, pinoresinol, and syringaresinol) glycosides (Heinonen et al. 2001) in the human gut by bacteria in the proximal colon (Aldecruetz, 2007). Lignans have been linked to the prevention of cancers and cardiovascular diseases (Begum et al. 2004). Lignans are believed to possibly possess properties to lower the risk of breast, prostate, and colon cancer as observed in epidemiological, animal, and *in vitro* studies (Webb and McCullough, 2005). Possible mechanisms for the cancer preventative abilities of lignans have been suggested to be from antioxidant, anti-estrogenic, pro-apoptotic, and anti-angiogenic properties (Webb and McCullough, 2005). A large prospective cohort study conducted to determine the risk of breast cancer in 58,049 postmenopausal French women compared the highest quartile of plant lignan consumption to the lowest (Touillaud et al. 2007). A significant 17% drop in relative risk was found for the highest quartile, with a range of

total plant lignans consumed being 1.36 – 5.70 mg/d (Touillaud et al. 2007). A case-control study consisting of 2,985 subjects investigated the effect of dietary lignans on colorectal cancer, comparing the highest and lowest tertiles of consumption (Cotterchio et al. 2006). The highest tertile of dietary lignan consumption (>0.255 mg/d) resulted in significant drop of 27% in colorectal relative risk compared to the lowest tertile (0 - 0.158 mg/d) (Cotterchio et al. 2006). In addition to these epidemiological studies, anti-carcinogenic effect of lignans have been found in rats consuming rye bran, pure lignans, and purified secoisolariciresinol glycoside (Adlercruetz, 2007). Secoisolariciresinol diglycoside levels of 0.05 - 0.2 g/kg supplemented in mouse diets gave a dose dependant drop in tumour growth, with the 0.2 g/kg dose level providing a significant drop in tumour growth compared to the control diet (Li et al. 1999). However, lignan consumed from wheat bran by a typical human male adult (wt 81 kg) based on the AI fibre level according to Health Canada (2010) would be 8.36 mg/d (See Table 2.2), which is ~ 2000 times lower than the amount that could significantly reduce tumour growth (0.2 g/kg x 81 kg = 16.2 g/d) as reported by Li et al. (1999).

Pettersson et al. (1996) using human ileostomy subjects reported that the amount of bacteria present in the small intestine was not sufficient to significantly alter plasma lignan levels even with diets high in plant lignans. A study by Lampe et al. (1999) revealed cereal grain dietary fibre intake and human urinary excretions of enterolactone and enterdiol over five days were significantly associated, although reported correlation values were very low ($r = 0.22$ ($P < 0.05$) and $r=0.34$ ($P < 0.01$), respectively). This association suggests

that lignins (composed of plant lignan subunits) possibly increase the amount of bioavailable mammalian lignans.

Begum et al. (2004) found lignin was a precursor for mammalian lignans in rat urinary excretions using wheat and rye bran. Urinary excretion of enterolactones (26-32%) was partly attributed to lignin and its involvement was confirmed by synthetic lignin production of enterolactones (Begum et al. 2004). In a different study, Frische et al. (2003) found a significant link between consumption of flaxseed (10 g/d), but not wheat bran (28 g/day) and lignan urinary excretion in sixteen premenopausal women. The different results for the mammalian lignan and bran relationship may be explained by the level of lignan consumed. In the rat study (Begum et al. 2004) had high levels of lignans corresponding to 10 - 15% of diet compared to 28 g/day of wheat bran (Begum et al. 2004; Frische et al. 2003). Lampe et al. (1994) reported significant increases of enterodiol and enterolactone in 18 premenopausal women consuming flaxseed at 10 g/day, which represents a major lignan source in diet, compared to a non-flaxseed diet. Urinary excretion levels of enterodiol and enterolactone increased from 1.09 to 19.48 μmol/L and 3.16 to 27.79 μmol/L (3 – 285 fold rise), respectively (Lampe et al. 1994). The duration of flaxseed consumption (3 months) and menstrual cycle did not affect the excretion levels (Lampe et al. 1994). This shows that flaxseed is able to greatly elevate mammalian lignans in the human body in comparison to low levels found with wheat bran.

2.9.2.6. Alkylresorcinols

Alkylresorcinols are phenolic lipid compounds that possess a phenolic ring, containing two hydroxyl groups attached at the meta position, with an odd numbered alkyl chain at position five. In cereals, the alkyl chain ranges from 15 to 25 carbons, which are mostly saturated. Alkylresorcinols are thought of as membrane antioxidants (Fardet et al. 2008). Alkylresorcinols have known to act as weak antioxidants *in vitro* compared to α-tocopherol through their hydrogen donating and radical scavenging abilities (Fardet et al. 2008). Their antioxidant potential depends on their chain length, which affects its amphiphilic feature and incorporation into cell membranes (Fardet et al. 2008). The strongest half maximal inhibitory concentration ($IC_{50}$) of erythrocyte membrane lipid oxidation was observed for C15:0 at a level of 10 μM (Kozubek and Nienartowicz, 1995). Larger chain lengths followed with values of 32.5 and 59.0 μM for C19:0 and C23:0, respectively (Kozubek and Nienartowicz, 1995). Alkylresorcinol's antioxidant ability may also be involved in protection of cells from carcinogenesis as reflected in Ames test results (Kozubek and Tyman, 1999). In this work, indirect acting mutagens, benzo[α]pyrene and 2-aminofluorene, at 10 μg/plate were inhibited by over 50% by alkylresorcinols (average 18.4 carbons and mostly saturated) (Kozubek and Tyman, 1999). Direct acting mutagens like methyl methanesulfonate and daunorubicin were less affected by alkylresorcinols, but inhibition was still apparent (Kozubek and Tyman, 1999). Since this compound is a relatively weak antioxidant, beneficial health effects *in vivo* would require high plasma

concentrations at levels that cereal grains could not contribute. However, membrane incorporation of alkylresorcinols could accommodate a biological effect given the concentration in cereals (Ross et al. 2004b). Alkylresorcinols consumed from wheat bran by a typical human male adult based on the AI fibre level according to Health Canada (2010) would be 203.07 mg/d (See Table 2.2). Limited data on alkylresorcinol's effective dose levels and wheat bran's high alkylrescorinol concentration calls for further studies on risk modulation of cancer and cardiovascular disease.

Alkylresorcinols are absorbed through the lymphatic system and have been recovered from rat adipose tissue, human lipoproteins (main transporter), human erythrocyte membranes (Linko et al. 2005), and human plasma (Ross et al. 2003a). Ross et al. (2003b) used rat models to study the recovery of alkylresorcinols in faeces, blood and urine. A pig model was also used in this study to determine ileal recovery levels. Rat models using radio-labelled alkylresorcinols single dosed at 4.6 mg/kg had recovery levels of 61% in faeces and 31% in urine (Ross et al. 2003b). Recovery of alkylresorcinols and metabolites in rat body tissues and urine was <1% and 30-54% (8% in the free form), respectively after over 100 hr (Ross et al. 2003b). Results suggested that alkylresorcinols are metabolized quickly and excreted in the urine (Ross et al. 2003b). Blood levels of radio-labelled alkylresorcinols in single doses of 5.0 mg/kg showed peaks at 7 and 12 hr after consumption, with most removed after 60 hr (Ross et al. 2003b). The elevated levels of intact free alkylresorcinols and metabolites recovered in the rat urine indicated that alkylresorcinol is highly bioavailable. Pigs fed rye grain/tissue diets containing alkylresorcinol levels in

whole-grain (484 mg/kg), aleurone (1290 mg/kg), pericarp-testa (204 mg/kg), and starchy endosperm (27 mg/kg) recovered levels of 37%, 40%, 21%, and 0%, respectively (Ross et al. 2003b). Absorption of alkylresorcinols in this pig model ranged from 60-79% depending on diet (Ross et al. 2003b). This result was similar to ~ 60% absorption seen in 10 human ileostomy subjects consuming high-fibre enriched rye bread (Ross et al. 2003a). That approximately 75% of alkylresorcinols were bioavailable in these studies indicates that apparently the indigestible bran matrix does not greatly, if at all, inhibit absorption. Results of upper intestine absorption of alkylresocinol in ileostomy subjects indicate that the source or dose level of alkylresorcinol affects or controls its absorption.

2.9.2.7. Phytic acid

Phytic acid is found in many foods high in fibre content (Shamsuddin, 2002; Somasundar et al. 2005), and wheat bran may be the most concentrated source (Table 2.2). Phytic acid is also, known as *myo*-inositol hexaphosphate ($InsP_6$ or $IP_6$), and comprises a simple carbohydrate ring with six phosphate groups attached to each carbon. Many animal studies have shown phytic acid to be capable of anti-neoplastic activities on multiple types of cancers of the breast, colon, liver, prostate, skin, and skeletal muscle (Fox and Ebert, 2002). Potential mechanisms suggested for phytic acid's anti-neoplastic properties include cell cycle inhibition, antioxidant ability (metal chelator), gene alteration, and increased natural killer cell activity (Fox and Ebert, 2002).

Phytic acid (PA) was able to prevent colon cancer tumour growth in a dose dependent manner for up to five months after ingestion by rats and mice (Vucenik and Shamsuddin, 2006). PA consumption (2% sodium phytate as liquid) one week after 30 mg/kg of azoxymethane (carcinogen) was injected subcutaneously significantly reduced occurrence of tumours in the colon of F344 rats after 36 weeks of consuming (Pretlow et al. 1992); tumour incidence in the control and PA treatment groups was 83% and 25%, respectively (Pretlow et al. 1992). Pre-existing liver cancers in mice were shown to regress after direct treatment of 20 mg/kg of PA (Vucenik and Shamsuddin, 2006). PA consumed from wheat bran by a typical human male adult (wt 81 kg) based on the AI fibre level according to Health Canada (2010) would be 3.22 – 4.65 g/d (Table 2.2), which 2 – 3 times the level that showed anti-carcinogenic effects as reported by Vucenik and Shamsuddin, 2006). This underscores the naturally high concentration of PA in wheat bran.

Phytic acid is believed to provide its efficacy for colon cancer prevention in relation to the gut epithelium (Fardet et al. 2008), and when absorbed in the blood, for CHD prevention (Ko and Godin, 1990). Sakamoto et al. (1993) determined that most of the absorption of phytic acid in rats took place in the stomach and upper small intestine (duodenum and jejunum); ~ 6% unabsorbed PA was found in the cecum and colon after 24 hr. Grases et al. (2001) in a clinical study reported that three PA supplements of varying levels (400 mg = calcium/magnesium salt, 3200 mg = calcium/magnesium salt and 1400 mg = sodium salt) had roughly the same absorption, as reflected by

urinary excretions. It appears there is an optimum PA intake in connection with maximum absorption in the GI tract, and that different forms of PA are absorbed similarly. The relationship of urine excretion and PA intake was examined by Grases et al. (2000) who used rats and a liquid PA diet in increasing concentrations (0, 61, 182, and 425 mg/L) in 38 ml/d per animal until excretion levels were constant. It was found that the 182 mg/L dose of PA gave the maximum urine excretion level, with excretion levels staying the same with increased phytic acid intake concentrations (Grases et al. 2000). A 182 mg/L phytic acid diet matches a 2% phytic acid intake (20.9 mg/kg body wt (rats)) which is likely at maximum absorption as noted (Grases et al. 2000). This maximum absorption appears to be very low which would equate to a low bioavailability of phytic acid in humans.

2.9.2.8. Phytosterols

Sterols are insoluble steroid-based alcohols that possess a hydrocarbon side chain about 8-10 carbons long at the 17β position and a hydroxyl group at the 3β position. Cholesterol is a 27-carbon sterol that can have a dietary source from animal products or is formed naturally primarily in the liver. Plant sterols or phytosterols differ from cholesterol by the addition of a methyl group, ethyl group, or additional double bond. Cholesterol, a hydrophobic compound, is required for intestinal absorption to be in a soluble form as dietary mixed micelles (DDM) (Rozner and Garti, 2006). Phytosterols are widely believed to possess intra-intestinal activity that inhibits cholesterol absorption, due to its

higher affinity to form DDM in the gut and the limited capacity of the GI tract to solubilise hydrophobic molecules (Ostlund Jr. and Lin, 2006). The low solubility and bioavailability of free phytosterol is believed to be the reason for requirement of large amounts (25-30 g/d) to be ingested in order to reduce cholesterol (Rozner and Garti, 2006). Inconsistent clinical studies regarding the cholesterol lowering ability of phytosterols have been attributed to the low bioavailability of the crystallized form (Ostlund Jr., 2002). A clinical study of phytosterols esterified to long chain fatty acids resulted in mean reduction of 9.6% of LDL cholesterol due to an intake of 2 g/day (Ostlund Jr., 2002). Phytosterols are believed to not significantly affect HDL or triglyceride concentrations (Rozner and Garti, 2006). Drug trial results have shown that a ~ 10% reduction of LDL cholesterol can cause CHD risk reductions of 12% to 20% over 5 years (Katan et al. 2003). Consuming corn oil containing phytosterols (150 mg per 30 g oil), compared to a phytosterol-free diet significantly reduced cholesterol absorption by 12.1 % (Ostlund Jr. et al. 2002). In another clinical trial where wheat germ (naturally high in phytosterols) containing muffins were consumed, it was found that pre-extracting phytosterols from wheat germ, compared to control muffins, resulted in a 42.8% increase in plasma cholesterol; wheat germ muffins contained 328 mg of phytosterols (Ostlund Jr. et al. 2003). The authors concluded that, "the efficiency of cholesterol absorption from test meals was substantially lower after consumption of original wheat germ than after consumption of phytosterol-free wheat germ." In that regard, phytosterol consumed from wheat bran by a typical human male adult based on the AI fibre level according to

Health Canada (2010) would be 1.14 – 1.41 g/d (Table 2.2), which is 4 - 9 times higher than the levels that produced cholesterol lowering effects from wheat germ and corn oil sources (Ostlund Jr. et al. 2003, Ostlund Jr. et al. 2002). However, there is a question regarding the bioaccessibility of phytosterols from wheat bran considering its highly indigestible nature.

Most of the absorption of plant sterols occurs in the duodenum or proximal jejunum (Dayspring, 2007). Phytosterols are absorbed to a lesser extent (< 2%) compared to cholesterol (close to 60%) (Rozner and Garti, 2006). As previously mentioned, phytosterols are commonly believed to function through intra-intestinal activity (Ostlund Jr. and Lin, 2006). Purified phytosterol crystals have slow solubility in bile salt solutions (requires days to weeks for solubilisation), due to their low lipid and water solubility (Ostlund Jr., 2002). New formulations of oil, margarine, and salad dressing can increase the solubility of phytosterol via esterification with fatty acids (Ostlund Jr. and Lin, 2006). Fatty acid esters are able to increase the solubility of β-sitosterol in fat (e.g. in oil and margarines) from 2.5% to 30% (Mattson et al. 1982). Esterification allows an increase in phytosterols relative to triglyceride in products (Ostlund Jr., 2002). These new formulations provided consistent and significant reductions in cholesterol in long-term studies (Ostlund Jr., 2002). Purified phytosterol crystals do not seem to be significantly bioavailable in the body due to their relatively low solubility, but corn oil and wheat germ muffin phytosterols show good bioavailability proven by their significant reductions in cholesterol absorption (Ostlund Jr. and Lin, 2006).

# 3. MATERIALS AND METHODS

## 3.1. Wheat bran samples

Bran of sound and representative samples of hard red spring common (hexaploid) wheat (CWRS) and durum (tetraploid) wheat (CWAD) wheat (2009 crop year) was prepared on a pilot Buhler mill (milling capacity 474 kg/h) at the Canadian International Grains Institute (Winnipeg, MB). CWRS wheat was tempered between 20 to 24 h with a milling moisture of about 16.5%. The CWAD wheat was processed for bread flour rather than semolina, and accordingly, a relatively long tempering time was used (72 h) in an attempt to mellow the very hard endosperm of durum wheat. Milling moisture for the CWAD wheat was between 16.5% and 17.0%. Resulting bran was produced at an extraction rate of about 76%. Coarse bran was collected as over tails of the final break roll of the mill; bran yield was approximately 11%. Commercial bran of sound soft white spring common (hexaploid) wheat (CWSWS) wheat was obtained from Horizon Milling-Cargill (Saskatoon, SK). Coarse bran (>2-3 mm) of all three types was subsequently processed on a Jacobson model 120B hammer mill (Carter-Day International Inc., Minneapolis, MN) to obtain fine bran (of similar particle size) to pass sieve openings of 1.17 mm (3/64 in). Bran samples were stored at -20 °C until processed.

3.2. Wheat bran treatment

Treated wheat bran (T) samples were 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). The total autoclave time was about 31 min, and included ~ 5 min temperature and pressure charging time prior to sterilization, and 10 min dying time. Non-autoclaved wheat bran is referred to as control wheat bran (C).

3.3. Wheat bran extractions

3.3.1. *In vitro* digestion extraction

*In vitro* digestion of wheat bran was carried out batch-wise, i.e. a static approach, with some modifications of methodology according to the works of Shen et al. (1994), Boisen and Fernandez (1997), Tedeschi et al. (2009), Toor et al. (2009), and Regmi et al. (2009). Those modifications included aspects of digestion time, digest reagents, pH targets and enzyme concentrations. Basically, a consensus method was adopted for this thesis research. The procedure is summarized in Fig. 3.1, and details were as follows:

1. The GA phase of the *in vitro* digestion:
   A. Ground wheat bran (100 g) was suspended in 37 ºC heated distilled water (800 ml).
   B. Hydrochloric acid (6.0 M) heated to 37 °C was used to lower the pH to 2.0. Pepsin solution (30 ml) heated to 37 °C which consisted of 0.391 g

of pepsin (Sigma P-7000) dissolved in 100 ml 0.1 M hydrochloric acid was added to the gastric digest. Distilled water heated to 37 °C was added to the sample digest to 900 ml. The digest was mixed on a magnetic stirrer in an incubator at 37 °C for 2.5 h.

C. If the digest was modeling the gastric phase only, then the digest solution was treated according to step 1.D. Otherwise the extraction continued with the small intestinal phase (step 2.A).

D. The GA digest suspension was filtered to separate insoluble hydrated bran from soluble extracts. This was done by vacuum assisted filtration of the digest using fiberglass mesh with nominal porosity of 1.1 mm.

E. The filtrate was centrifuged at 3,550 x g to obtain a clear supernatant which represents the soluble GI digest extract.

F. The supernatant was transferred to a lidded plastic container with ample surface area to accommodate efficient freezing (-35 $^{o}$C) and subsequent freeze-drying. Insoluble residue material after centrifugation was added to the branny retentate (insoluble digest material), and was likewise frozen and freeze-dried.

G. If the supernatant after the centrifugation step appeared cloudy, the solution was filtered using a Buchner funnel and Whatman filter paper #41.

2. The small intestinal, i.e. GI phase of the *in vitro* digestion:

A. Sodium hydroxide (3.0 M) heated to 37 °C was added to the gastric digest to adjust the pH to 7.0. Distilled water was added for a total

volume of 950 ml at this stage, and the mixture was incubated for 30 min at room temperature on a magnetic stirrer.

B. Pancreatin extract solution (50 ml) was added to the digest. The solution was made from 1.90 g pancreatin (Sigma P-1750) mixed in 27 ml of distilled $H_2O$ for 20 min, and then centrifuged at 2800 x g. The supernatant of the pancreatin extract was made up to 50 ml with distilled water heated to 37 °C.

C. The incubation period for the small intestinal digestion was continued for a total of 4.5 h at 37 °C on a magnetic stirrer in an incubator.

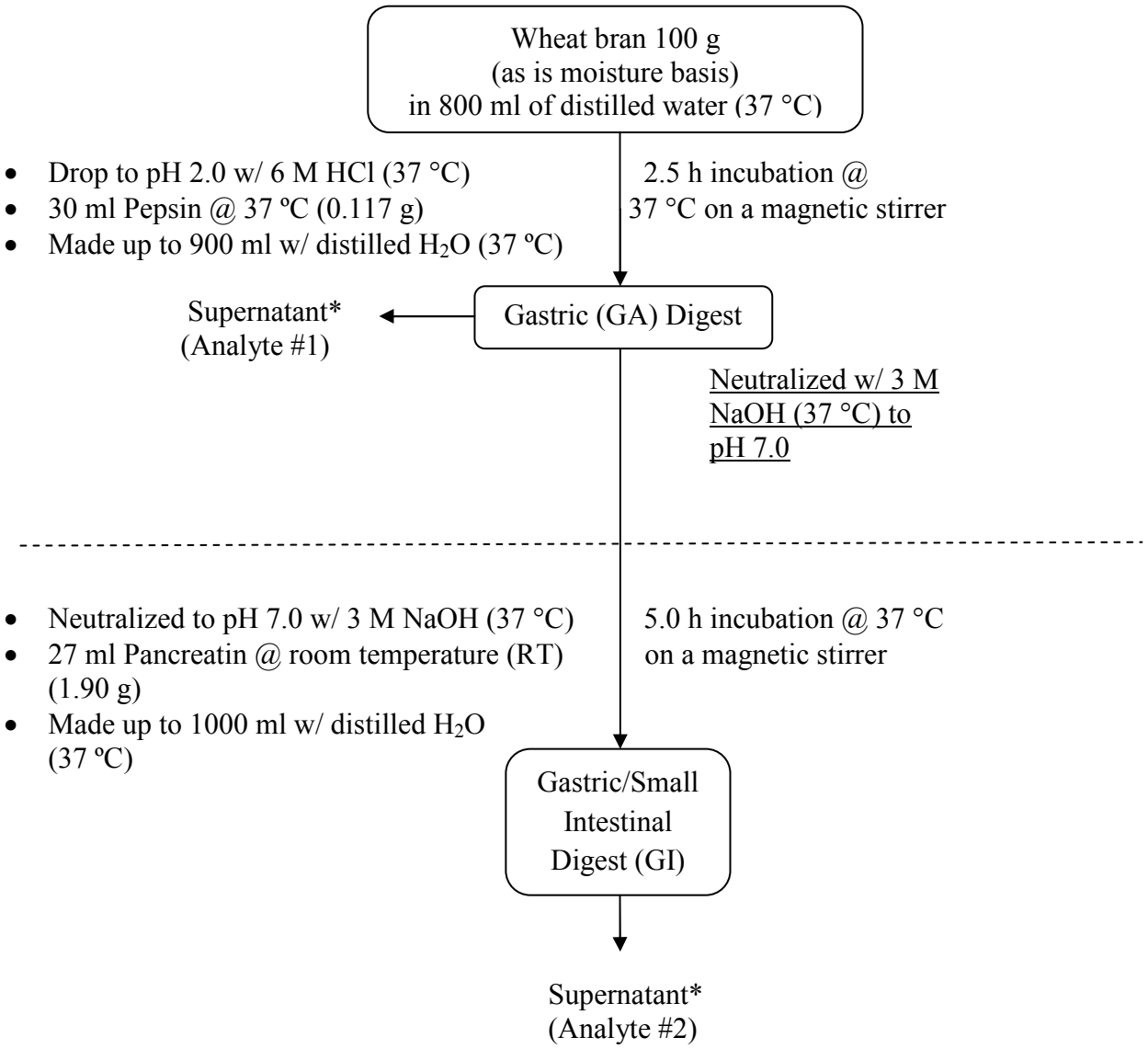


Figure 3.1. Wheat bran *in vitro* digestive process

*Using vacuum assisted filtration the digest solution was poured through fiberglass mesh (1.1 mm), and then the filtrate was centrifuged @ 3,550 x g for 10 min and then freeze dried. If suspended solids still did not precipitate, the digest was put through a vacuum assisted Buchner funnel using Whatman filter paper no. 41.

### 3.3.2. Water extractions

Water extractions of wheat bran had the same ratios of sample to liquid and incubation periods as the *in vitro* digests. The water extractions had a neutral pH throughout, and used no enzymes.

## 3.4. Chemical Analysis of Wheat bran and Wheat Bran Digests

All analytical determinations were expressed on a dry basis.

### 3.4.1. Moisture analysis

Moisture analysis was performed according to AOAC Official Method 925.10 with some modifications. Samples (0.2 g) was weighed in metal dishes and placed in air oven set at 130 ºC for 1 h. After drying the sample was covered the dish lid, placed in dessicator, and weighed after reaching RT. Moisture content was determined as loss in weight after drying.

### 3.4.2. Sodium and other mineral analysis

Due to the titration of GA digests with NaOH, salt is a significant by-product that needed to be accounted for in the analysis of digest composition. Sodium and other mineral analysis of GA and GI samples, as well as selected brans was performed by inductively coupled plasma-optical emission

spectroscopy (ICP-OES) using facilities at the Brandon Research Centre, AAFC (Dr. Cynthia Grant). Sample (0.5 g) was oxidised in 10 ml 4% (v/v) nitric acid (trace metal grade) using a CEM Microwave, MARS Express system (CEM Corp., Matthews, North Carolina) in 75 ml digestion vessels. Digestion was carried out in the 1600 W microwave digester in two stages: 1) linear gradient from ambient to 200 $^{o}$C over 20 min, and 2) holding the sample at that temperature and 120 psi (absolute) for 15 min. Digests were subsequently diluted to 37.5 ml with 18.2 MOhm water. Digests were analyzed using a Perkin Elmer DV 5300 ICP-OES instrument. Sodium and non-sodium elements were analyzed separately. Sample uptake was 1.0 ml/min. Visible spectrum emission lines were monitored in radial view through the plasma, except for sulfur. Emission lines were as follows: Na in GI and GA digests: 330.237 nm, Na in bran: 589.598 nm, Fe: 259.938 nm, K: 766.499 nm, Mg: 279.078 nm, Mn: 257.610 nm, P: 213.622 nm, Ca: 317.934 nm, Cu: 327.399 nm. Sulfur was monitored using an ultraviolet detector at 180.673 nm. NaCl content of samples was calculated using the analyzed concentration of sodium and the mass proportion of the chlorine ion using atomic weights of Na and Cl of 22.990 and 35.453, respectively.

### 3.4.3. Ash content

Ash analysis was performed according to AOAC Official Method 923.03 with some modifications. Samples (0.5 g) was weighed in ashing crucibles and ignited in the ashing oven at 550 ºC overnight (16 h) and after

reaching 100 ºC placed in a dessicator and subsequently weighed when RT was achieved. The loss in solids was reported as loss in weight due to moisture. Ash content was determined as weight remaining after heating.

### 3.4.4. Protein content

Total nitrogen of bran and digests was determined by combustion nitrogen analysis using a Dumas (LECO Model FP-428, St. Joseph, MI) CNA Analyser (Sweeney and Rexroad, 1987). A factor of 6.31 was used to convert total nitrogen to protein (N to P) content (Jones 1941, FAO/WHO, 1970).

### 3.4.5. Total starch content

Total starch content of the wheat bran samples was determined according to the Megazyme Total Starch Assay Procedure (Amyloglucosidase/α-Amylase Method) AOAC Method 996.11. Wheat bran samples (0.1 g) ground to pass a 0.5 mm screen was suspended in 5 ml of 80% v/v ethanol and incubated for 5 min in 85 ºC water bath, then vortexed. Another 5 ml of 80% v/v ethanol was added and then vortexed. The tube was centrifuged at 1000 x g for 10 min using a benchtop centrifuge. The supernatant was decanted and an additional 10 ml of 80% v/v ethanol and centrifuged at 1000 x g for 10 min. The supernatant was decanted then 3 ml of thermo-stable α-amylase in MOPS buffer (300 units; 50 mM, pH 7.0) added and then vortexed. The tube was then incubated in a boiling water bath for 6

min (vortexing at the 2 and 4 min mark). Sodium acetate buffer (200 mM, pH 4.5) was added (4 ml) to each tube along with amyloglucosidase (0.1 ml). The tube was then vortexed and placed in 50 ºC water bath and incubated for 30 min (vortexed at 15 min). Samples that contained over 10% starch were diluted to 100 ml in volumetric flasks with distilled water (mixed thoroughly) and then 10 ml of the solution was added to tubes. Samples containing less than 10% starch were filled to 10 ml with distilled water without dilution. The tubes were then centrifuged at 1550 x g for 10 min. The supernatant (0.1 ml) was added to culture tubes for each sample. Megazyme glucose control was added (0.1 ml) to four tubes and distilled water (0.1 ml) was added to one tube as the reagent blank. GOPOD (3 ml) was added to all the tubes and then the tubes were immediately incubated in a 50 C water bath for 20 min. The absorbance of each sample and glucose control was read against the reagent blank at 510 nm. Analysis was conducted in duplicate.

Calculations:

*Samples containing over 10% Starch*:

Total Starch % = E x F/W x 90

*Samples containing less than 10% Starch*:

Total Starch % = E x (F*10/W) x 90

Where E = Absorbance read against the blank, F = (100/Absorbance of 100 µg of glucose, from glucose control), W = weight of flour analyzed (mg), and 90 = conversion factor

### 3.4.6. Total pentosan content

Total pentosan content was determined using two very similar methods. The Douglas method is used for samples containing very little fibre, while the Bell method includes a pre-digestion step for samples containing high-fibre.

#### 3.4.6.1. Douglas method

Total pentosan content analysis for wheat bran digests was performed according to the colorimetric phloroglucinol method of Douglas (1981). D-(+)-Xylose was used as a standard to construct calibration curves. Pentosan content was calculated according to the method based on the absorbance differences at 552 and 510 nm using a calibration curve. Wheat bran extract (0.005 g) or a control blank $H_2O$ solution (2 ml) was placed in 15 ml test tubes. The colorimetric reaction reagent (10 ml) was added to the tube and vortexed. This reagent was comprised of phloroglucinol (1 g), 95% ethanol (5 ml), acetic acid, AR, glacial (110 ml, HCl, AR, concentrated, 2 ml, and glucose, 1.75% w/v, 1 ml. The tubes were then incubated in a boiling water bath for 25 min. After cooling the tubes for 5 min in an ice water bath, the absorbance of the solution was measured at 552 nm and 510 nm. Xylose was used as the standard with concentrations ranging from 0 – 0.02 mg/ml. A standard curve determined for the results was expressed in terms of % pentosan.

3.4.6.2. Bell method

Total pentosan content analysis for wheat bran was performed according to Bell (1985). Wheat bran (0.200 g) was placed in 15 ml test tubes. For a complete release of pentosan from the bran material, each wheat bran tube was filled with 0.5 M sulphuric acid (10 ml) and vortexed. The tubes were then placed in a boiling water bath for 30 min with marbles covering the tubes. After the boiling water bath, the tubes were cooled for 15 min at room temperature. The tubes were centrifuged at 8,000 x g for 10 min. For bran samples the supernatant was diluted by 100 times in 100 ml volumetric flasks. Wheat bran or control samples (2 ml) were then placed in 15 ml test tubes. Thereafter, the method of Douglas (1981) was applied to determine pentosan content expressed as % pentosan.

3.4.7. Total β-glucan content

Total β-glucan content of the wheat bran samples was determined according to the Megazyme Mixed-Linkage Beta-Glucan Assay procedure (McCleary Method) AOAC Method 995.16. Wheat bran and digests samples (0.08 – 0.1 g) ground to pass a 0.5 mm screen was weighed into tubes, with a mini stirring bar added. The samples were then suspended in 0.2 ml of aqueous ethanol (50% v/v) and 4 ml of sodium phosphate buffer (20 mM, pH 6.5), then vortexed. The tubes were then incubated in a boiling water bath for 3 and allowed to stir on a low setting on a magnetic stirrer. The tubes were then

incubated in a 50 ºC water bath for 5 min. 0.2 ml of lichenase was added to the tubes, then vortexed. The tubes were then placed in a 50 ºC water bath for 60 min, with vortexing taking place in 12 min intervals. 5.0 ml of 200 mM acetate buffer was added to the tubes and vortexed. The samples were then centrifuged at 1000 x g for 10 min and 0.1 ml of the supernatant was placed in three test tubes. 0.1 ml of β-glucosidase was added to two of the tubes and 0.1 ml of 50 mM acetate buffer (pH 4.0) was added to the third tube as the blank. All tubes were then incubated in a 50 ºC heated water bath for 10 min. Two Megazyme glucose standards were included (0.1 ml of glucose standard plus 0.1 ml of 50 mM acetate buffer (pH 4.0)). A reagent blank was included, which contained 0.2 ml of 50 mM acetate buffer (pH 4.0). Tubes were removed from the water bath and 3.0 ml of GOPOD was added to the sample, standard, and blank tubes. All the tubes were incubated at 50 ºC for 20 min. Absorbance was read at 510 nm within an hour after incubation. The sample blank was recorded and subtracted from the readings of samples before making the following % β-glucan calculations:

% β-glucan: $\Delta E \times F/W \times 8.46$

$\Delta E$ = absorbance minus treatment blank
F = 100 µg glucose/GOPOD abs for 100 µg glucose
W = weight of sample in mg
8.46 = conversion factor

3.4.8. Soluble and Insoluble Fibre Content

Soluble and insoluble fibre content of the wheat bran samples was carried out according to the Megazyme Total Dietary Fibre Assay Procedure AOAC 991.43. A blank (no sample) and a check (oat bran) sample were included the analysis. Wheat bran samples (1.0 g) ground to pass a 0.5 mm screen weighed into beakers was suspended in 40 ml of MES-TRIS blend buffer solution (0.05 M, pH 8.2) at 24 ºC and 0.05 ml of heat-stable α-amylase. The beakers were then incubated in a 95-100 ºC water bath for 35 min. Beaker sides were scraped and rinsed with 10 ml of distilled water. Protease (0.1 ml) was added to each beaker and then they were placed in a 60 ºC water bath for 30 min. HCl (0.561 M) was added to each beaker to reach a pH of 4.1 – 4.8 and amyloglucosidase (0.2 ml) was added. The beakers were then incubated for another 30 min in a 60 ºC water bath before the filtering step. Analysis was conducted in quadruplicate.

<u>Insoluble fiber (IDF) content</u>

Crucibles weighed to 1.0 g with celite that was dried (130 C oven) the night before was placed on filtration flasks and preheated distilled water (70 C) was used to wet distribute the bed of celite, while applying suction. Each sample beaker was then poured into each crucible for and preheated distilled water (20 ml) was used to rinse the residue into the crucible. The filtered solution was transferred to a glass container for soluble fibre determination. The crucible then was washed with 20 ml each of 95% v/v ethanol and 100%

v/v acetone. Finally, the crucible was dried overnight in a 103 ºC oven. After cooling the crucibles in a dessicator one crucible's celite and residue was ground for ash analysis and the other was ground for protein analysis (LECO FP-528 instrument, N to P conversion factor = 6.25).

Soluble fiber (SDF) content

The preserved filtrate from the IDF filtration was combined with 4 vols of 95% v/v ethanol that was preheated to 60 ºC. Precipitate was allowed to form at room temperature for 60 min. Crucibles weighed to 1.0 g with celite that was dried (130 ºC oven) the night before was placed on filtration flasks and 78% v/v ethanol was used to wet distribute the bed of celite, while applying suction. The precipitated solution was filtered through the crucible and 78% v/v ethanol was used to transfer all remaining particles to the crucible. The residue was then washed with 30 ml each of 78% v/v ethanol, 95% v/v ethanol, and 100% v/v acetone. Finally, the crucible was dried overnight in a 103 ºC oven. After cooling the crucibles in a dessicator one crucible's celite and residue was ground for ash analysis and the other was ground for protein analysis (LECO FP-528 instrument, N to P conversion factor = 6.25).

CALCULATIONS:

$$\text{Dietary Fibre (\%)} = \frac{((R_1 + R_2)/2 - p - A - B)}{(m_1 + m_2)/2} \times 100$$

where:

$R_1$ = residue weight 1 from m1
$R_2$ = residue weight 2 from $m_2$
$m_1$ = sample weight 1
$m_2$ = sample weight 2

A = ash weight from $R_1$ p = protein weight from $R_2$
B = blank

Blank, $B = (BR_1 + BR_2)/2 – BP - BA$

where:

BR = blank residue BP = blank protein from $BR_1$
BA = blank ash from $BR_2$

3.4.9. DPPH radical scavenging activity

DPPH radical scavenging activity method was performed according to Cheng et al. (2006), with some modifications. Wheat bran (0.1 g) ground to pass a 0.5 mm screen was extracted in 1 ml of 50% v/v acetone by initially vortexing thoroughly and then for 2 hr on a rotary shaker (RKVSD rotor mixer, ATR Inc., Laurel, MD) at 20 rpm). The microcentrifuge tubes were then centrifuged at 3,180 x g for 10 min. The absorbance at 515 nm of 0.208 mM DPPH solution for each sample was measured before sample addition to test tubes. After measuring the initial absorbance, the supernatant (0.1 ml) for each sample was added to 3.9 ml of 0.208 mM DPPH solution and vortexed. The solution was then stored at room temperature for 40 min and the absorbance at 515 nm was measured again. Trolox standards were prepared ranging from 0 - 2 mM. The solutions and incubation periods were all conducted in a dark room. A standard curve was prepared from the trolox standards and results were expressed in terms of trolox equivalents (TE μmol/g of sample). Analysis was conducted in duplicate.

3.4.10. Iron metal chelating activity

Iron metal chelating activity method was performed according to Xie et al. (2008), with some modifications. Wheat bran samples (0.015 g) ground to pass a 0.5 mm screen were first vortexed thoroughly and then extracted in 1.5 ml of distilled water for 2 hr on a rotary shaker (20 rpm) in micro-centrifuge tubes. The tubes were then centrifuged at 10,687 x g for 10 min. In each test tube 0.1 ml of supernatant, 0.05 ml of iron dichloride solution (2 mM), 2.75 ml of distilled water, and 0.1 ml of Ferrozine (5 mM) was added, then vortexed. The prepared standard curve for $Na_2$-EDTA contained 1 ml of $Na_2$-EDTA (0 - 125 µM), 0.05 ml of iron dichloride solution (2 mM), 1.85 ml of distilled water, and 0.1 ml of Ferrozine (5 mM). After solution stood at room temperature for 10 min the absorbance at 562 nm was measured. A standard curve was determined for $Na_2$-EDTA and the results were expressed in terms of $Na_2$-EDTA equivalents ($Na_2$-EDTA µmol/g of sample). Analysis was conducted in duplicate.

3.4.11. Total phenolic content

Total phenolic content analysis was performed according to Gao et al. (2002), with some modifications. Wheat bran extracts (0.03 g) in micro-centrifuge tubes was extracted in 100% methanol (1 ml) for 0.5 hr and centrifuged for 10 min at 3,180 x g. The supernatant or control (0.025 ml) was added to 10% v/v Folin Ciocalteu reagent (0.75 ml), and allowed to equilibrate

for 5 min at room temperature. After allowing the tubes to stand for 5 min, 0.75 ml of sodium carbonate (60 g/L) was added. Absorbance at 725 nm of the final solution was measured after 90 min of incubation at room temperature. The ferulic acid control used ranged from 0 - 1 μM. A standard curve was determined for ferulic acid and the results were expressed in terms of ferulic acid equivalents (FA μmol/g of sample). Analysis was conducted in duplicate.

3.4.11. Experiment design and statistical analysis

Statistical analysis was performed using SAS software (v.9.2, SAS Institute, Cary, NC). The basic design of the study involved preparation of soluble digests from representative brans of three Canadian commercial wheat classes without and with autoclave thermal treatment. Specifically, digest material for analysis was generated from three brans (hard, soft and durum wheat), subjected to two thermal treatments (untreated control and roasted as described previously), by application of two standardized digestion treatments simulating GA and GI *in vivo* conditions. Therefore 12 combinations of "factors" were assessed for concentration and yield of a range of soluble analytes in each digest. Data collection followed a randomized design. All analytical tests were conducted using at least two independent determinations or subsamples. The significance of digestion phase (GA vs. GI) within each thermal treatment (C vs. T) was initially determined by conducting a one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure to estimate variance. As mean differences in analyte yield between digestion treatments was invariably found to be large (except SDF) (and much

larger than effects due to bran and thermal treatment) and highly significant (refer to Table A.9 & A.10), all subsequent statistical analyses were carried out by digestion type, and mean differences were assessed using Fisher's least significant different (LSD) method at $P < 0.05$ level.

In order to more fully evaluate the digestion method used, a separate experiment using control and autoclaved CWRS bran was performed. This was done to compare the effects of digestion with and without pepsin and pancreatin for GA and GI digests, respectively, and also to compare those results with simple water extracts of wheat bran. The rationale for this was to provide a comprehensive analysis of digestion effects. Results of this experiment were analyzed using a one way ANOVA using the GLM procedure. Mean differences were evaluated using Fisher's LSD.

# 4. RESULTS

## 4.1. Dry matter yield

### 4.1.1. Dry matter yield of CWRS wheat bran digests and extracts

Freeze dried product of extracts and digests of wheat brans provides an indicator of wheat bran digestibility. The dry matter (DM) yield of *in vitro* digests and water extracts of CWRS wheat bran ranged from 10.3 to 28.1 g/100 g bran (Fig 4.1A). On the lower end of the range were the 2.5 and 7.5 h water extractions. Water extracts of autoclaved bran had the lowest dry matter yield. The control bran water extracts were consistently higher in yield compared to autoclaved bran. The water extraction times of 2.5 and 7.5 h did not appear to be significantly different, although higher dry matter yields appeared to be associated with the longer extraction time. The lowest DM yield for digests was for the GA-no pepsin control sample, which was similar to results for the control water extracts. This outcome points to a minimal effect of low acid pH treatment on wheat bran digestibility since a water extraction at approximately neutral pH gave comparable amounts of DM. The efficacy of including pepsin in the digestion procedure is well shown (Fig. 4.1A) as the GA control digest with pepsin resulted in approximately 20% increase in DM yield. The DM content of GA digests for control vs. autoclaved brans appeared to be different as will be discussed in the following section. Among the CWRS GI digests, the lowest DM yield was found for the GI-no pancreatin control digest. The CWRS GI-no pancreatin control digest was similar in DM yield to the GA

control digest and was approximately 30% lower than the GI control digest containing pancreatin. The enhancement of DM yield for the GI digest with pancreatin clearly demonstrates the effect of enzymes such as α-amylase, protease and possibly lipase to increase the digestibility of wheat bran. The DM yields of digests of GI control vs. autoclaved brans were not different.

4.1.2. Dry matter yield of *in vitro* digests of three wheat brans

The order of DM yields of GA and GI digests of the different brans, whether control or autoclaved, were as follows: CWAD > CWSWS > CWRS (Fig 4.1.B). This ordering appeared to be closely associated with the protein and starch contents of the brans, as both of these constituents, as will be shown later, were relatively highly concentrated in bran and both had relatively high digestibilities. Accordingly, brans with higher levels of starch and protein (i.e. CWAD and CWSWS brans) had higher overall DM yields compared to that of CWRS wheat bran.

For GA digests of control brans, DM yields were in the range of 18.6 to 23.6%. Much higher DM yields were found for GI digests which were in the range 28.1 to 47.9%. A trend among all the wheat brans was the higher DM yields for GA digests of control vs. autoclave treated brans. DM yields of control GA digests of CWRS, CWSWS, and CWAD were 18.6%, 22.2% and 23.6%, respectively. By comparison, the corresponding DM yields of the autoclaved GA digests were 15.6, 17.5, and 19.7%, respectively. For the GI

digests, DM yield differences between corresponding control and autoclaved brans appeared to be smaller or non-existent.

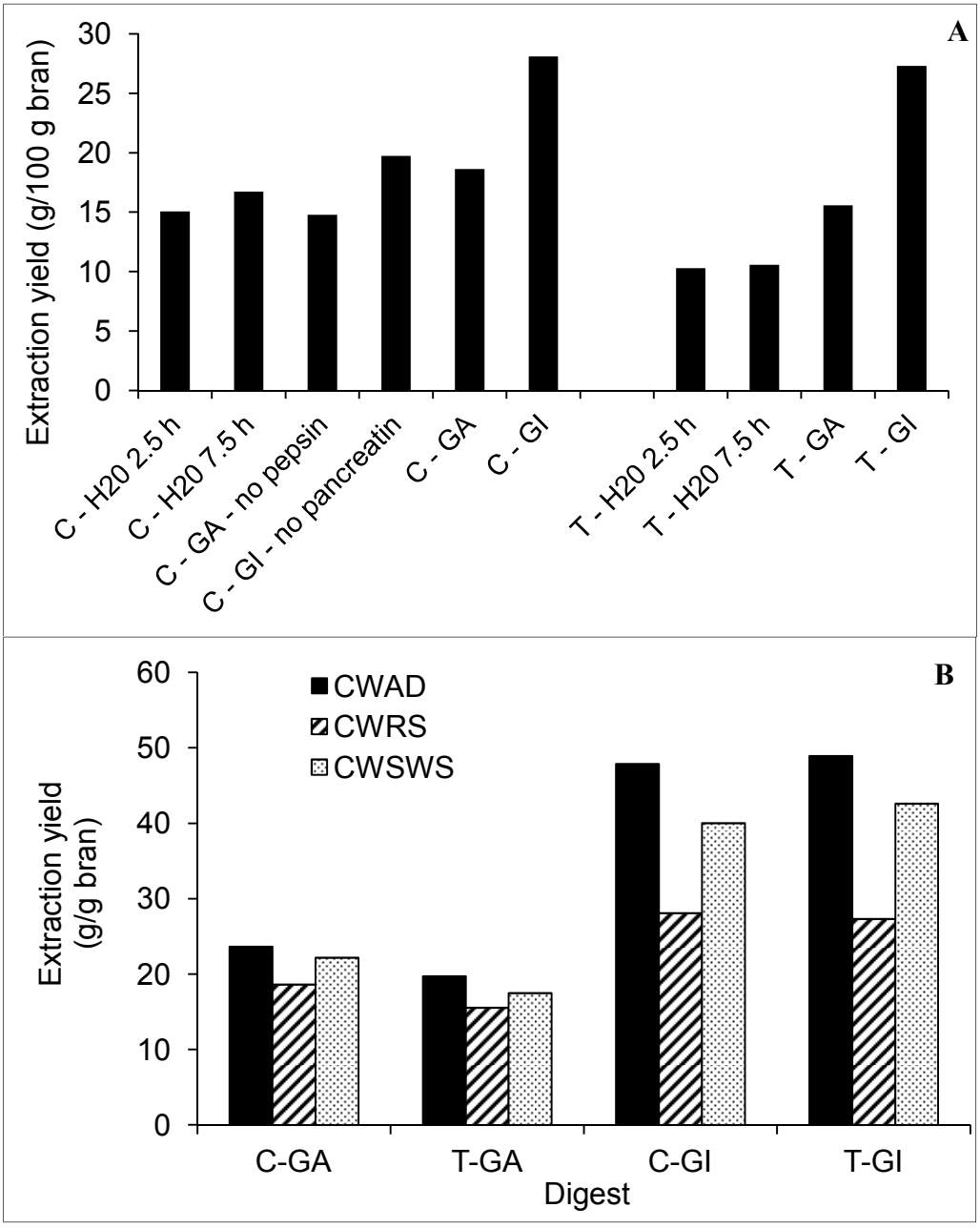


Figure 4.1. Dry matter (DM) yields of bran digests and water extracts. CWRS bran results are shown in (A) for control (C) and autoclaved (T) bran from gastric (GA) and gastrointestinal (GI, includes GA phase) *in vitro* digests. Results also shown in (A) for GA digests without pepsin, GI digests without pancreatin, and water (H2O) extracts of bran for indicated times. DM results for *in vitro* digests of three brans are shown in (B).

## 4.2. NaCl content of wheat bran digests

Depending on the extent of titrations, considerable quantity of salt can be formed when GA digests at pH 2.0 are neutralized with NaOH to simulate GI digestion. All DM yields of wheat bran digests were increased by NaCl produced from the acid-base titration occurring in the digestion procedure. Accordingly, there was a need to quantify the NaCl content of the digests in order to obtain accurate concentrations of other analytes due to the *in vitro* digestion. The NaCl concentration in bran digests ranged from 6.4 to 18.4% (Fig 4.2). No apparent differences in NaCl yield was found in digests between control and autoclaved brans, but a difference was apparent when comparing GA vs. GI digests (Fig 4.3). Averaging across all brans, the mean NaCl content of GA and GI digests were 3.4 and 3.7 g/100 g bran, respectively. The higher NaCl content of GI digests likely arises from greater breakdown of fibre occurring over the longer incubation time of the GI phase which would result in greater release of minerals (including salt) bound to insoluble fibre. Pentosan and β-glucan results presented below appear to affirm this suggestion.

## 4.3. Concentration and yield of bran digest constituents

Analysis of *in vitro* digests of wheat bran for various constituents was determined on a (freeze dried) DM basis. Concentration results were corrected to account for NaCl content of digests arising from NaOH titrations as explained previously. Analyte yield results reported per g (bran) basis required no similar data correction. Bioaccessibility results are reported as extractability

of a given constituent in dried digest as a percentage of that analyte in the original bran corrected to dry basis.

It should be noted that concentration results for analytes in digests are of limited value in interpreting the effects of different digests, brans, thermal treatment, etc. This is because the concentration of a given analyte is affected by the aggregate concentration of all other analytes in the sample. As an example, ash (total mineral) concentration in the 2.5 h water extract of control CWRS wheat bran was 18.3%, whereas the concentration of ash in the GI digest of control CWRS bran was 11.5% (refer to Fig. 4.4A). Because GI digests contain considerably greater amounts of other extractable compounds from wheat bran, the concentration of ash is lower than that for simple water extracts where ash represents a higher overall proportion of soluble compounds. Analyte yield values and bioaccessibility percentages are much better parameters for gauging the effects of *in vitro* digestion. Accordingly, in this chapter, while concentration data are documented in parallel with yield results, the former receive little comment.

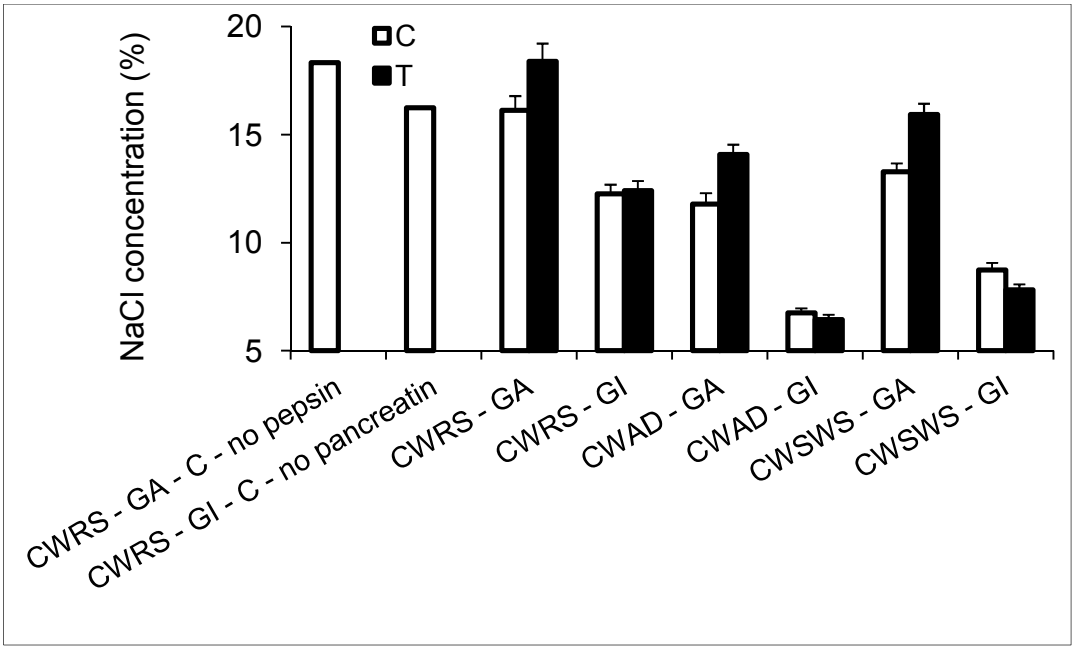


Figure 4.2. NaCl concentrations of *in vitro* gastric (GA) and gastrointestinal (GI) digests for control (C) and autoclaved wheat bran (T). NaCl concentrations of CWRS bran GA digests without pepsin and GI digests without pancreatin are also shown.

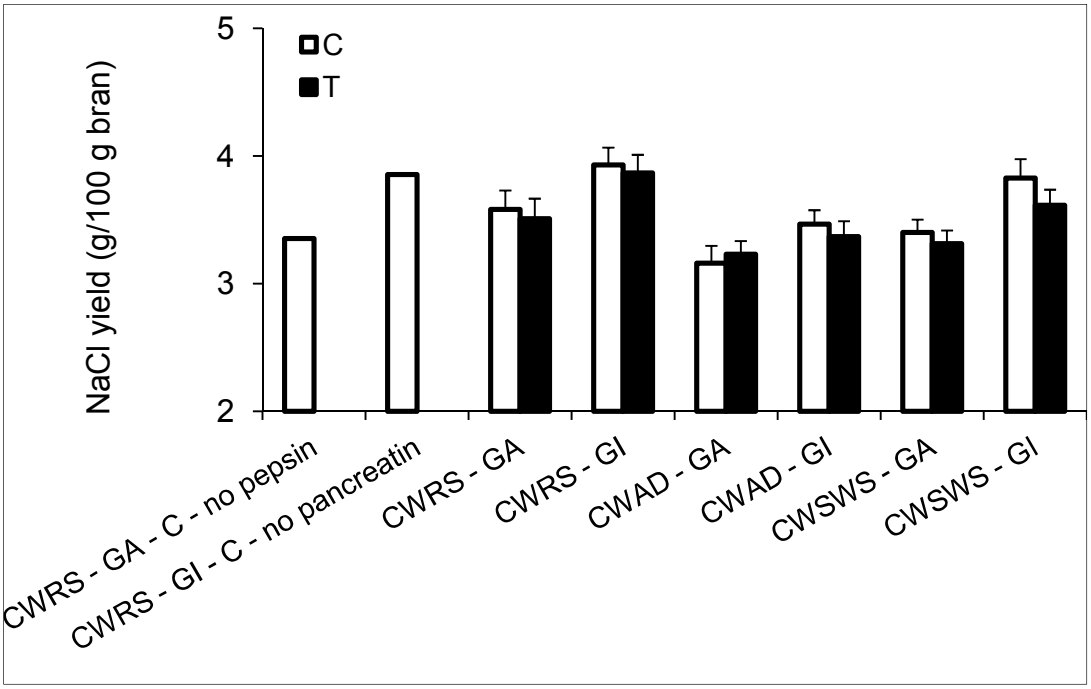


Figure 4.3. NaCl yields of *in vitro* gastric (GA) and gastrointestinal (GI) digests for control (C) and autoclaved wheat bran (T). NaCl yields of CWRS bran GA digests without pepsin and GI digests without pancreatin are also shown.

4.3.1. Ash content of the three wheat brans

Concentration of ash in CWAD and CWSWS wheat brans were similar and averaged 5.5 and 5.6%, respectively. CWRS wheat bran had significantly higher ash content at 7.7% (Table 4.1). There was no significant difference in ash content of brans due to autoclaving (results not shown). The higher ash concentration in CWRS wheat bran compared to the other brans may be due to the considerably lower starch content of CWRS bran (to be discussed later) which had the effect to increase the relative concentration of minerals (i.e. ash) in the bran.

4.3.1.1. Ash content of CWRS bran digests and water extracts

Ash concentration of CWRS bran digests and water extracts ranged from 11.6% to 22.0% depending on the specific digest or sample (Fig. 4.4A). Interestingly, treatments with the highest concentration of ash were the simple water extracts of control bran which were significantly higher in ash concentration compared to other digests and water extracts of autoclaved bran. This outcome arises most likely because of significant increases in concentration of other nutrients and phytochemicals when digests were simply acidified (GA-no pepsin), or when enzymes were added as part of the digestion procedure, or even for water extracts of autoclaved bran, which would in turn reduce the relative concentration of ash extracted as reflected in concentration results.

In terms of yield of ash for CWRS wheat bran, the highest ash yield was found for the GI digest of autoclaved bran (GI-T) and the lowest was 2.5 and 7.5 h water extracts of autoclaved bran (Fig 4.4B). Both the 2.5 and 7.5 h water extracts of control bran were significantly and considerably (more than 2X) higher in ash yield than their autoclaved bran counterparts. Ash yields of GA and GI digests of autoclaved bran were significantly higher than corresponding 2.5 and 7.5 h water extracts by 63.5 and 67.1%, respectively. GA digests of control bran (30.5 mg/g) were significantly higher in ash yield compared to corresponding 2.5 h water extracts (24.6 mg/g). *In vitro* GA and GI digests of bran without pepsin and pancreatin, respectively, had slightly lower ash yields compared to corresponding GA and GI digests with these enzymes.

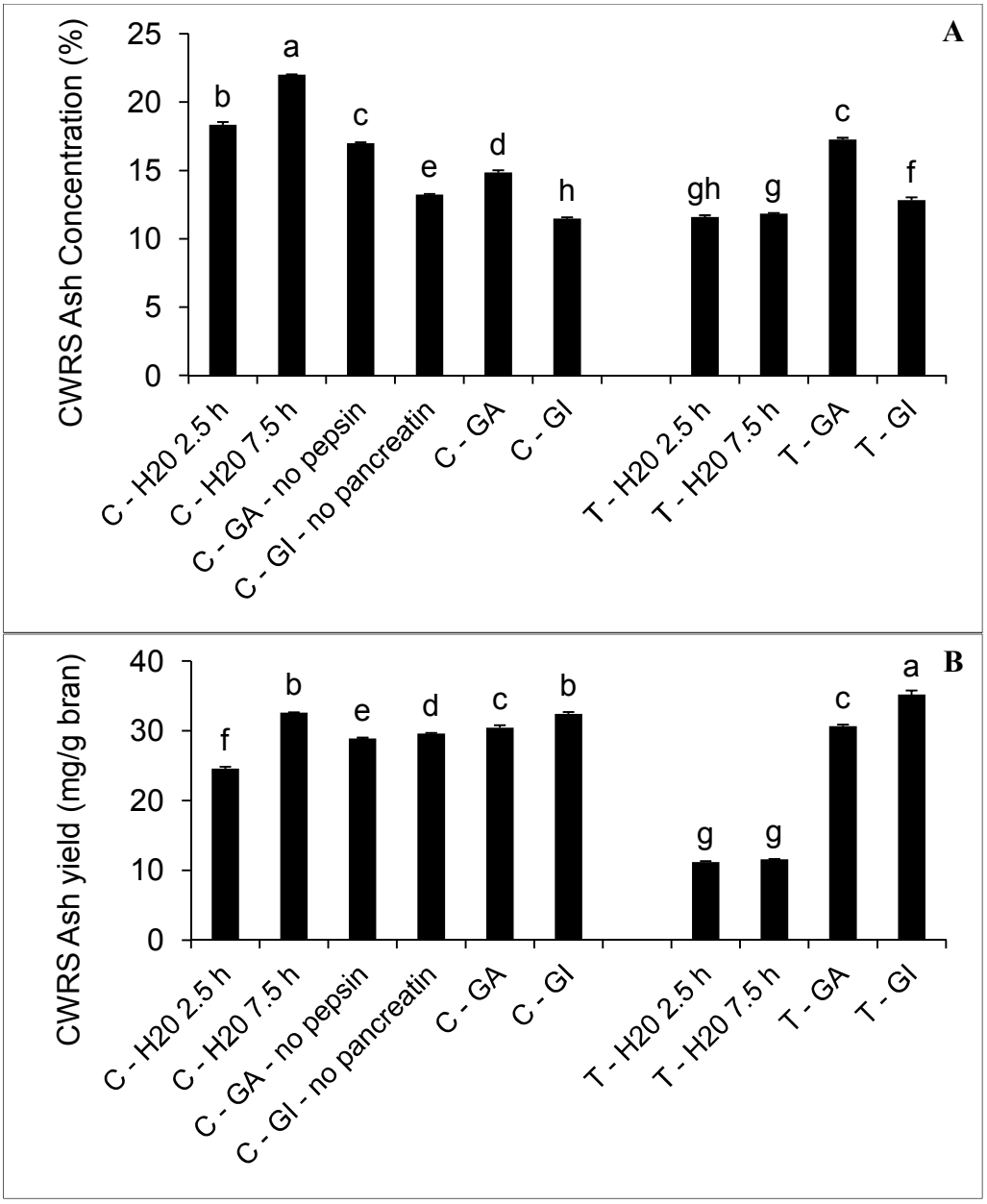


Figure 4.4. CWRS ash concentration (A) and yield (B) of *in vitro* gastric (GA) and gastrointestinal (GI) digests for CWRS wheat brans of control (C) and autoclaved wheat bran (T). Results also shown for GA digests without pepsin, GI digests without pancreatin, and water (H2O) extracts of bran for indicated times. Bars with different letters are significantly different ($P < 0.05$).

4.3.1.2. Ash content of *in vitro* digests of the three wheat brans

It can be seen from visual inspection of Fig. 4.5 that concentrations and yields of ash in *in vitro* digests of wheat brans followed a similar pattern of variation with CWRS bran digests having the highest values and CWAD brans having the lowest within a digest type. Yields of ash across digests ranged from 22.5 to 35.2 mg/g bran. For a given bran type, ash yields of GI digests were significantly higher by 13.7% on average, than those for GA digests. The longer time frame of GI digestions and not the presence of pancreatin enzyme most likely accounts for the increase.

Comparing the effects of autoclaved bran vs. control bran in GA digests; the only significant difference was for CWSWS bran which had lower ash yield (25.2 mg/g bran) compared to control bran (28.8 mg/g bran). In GI digests there was a different trend, as both CWAD and CWRS brans were slightly, but significantly, higher in ash yield for autoclaved bran compared to control bran.

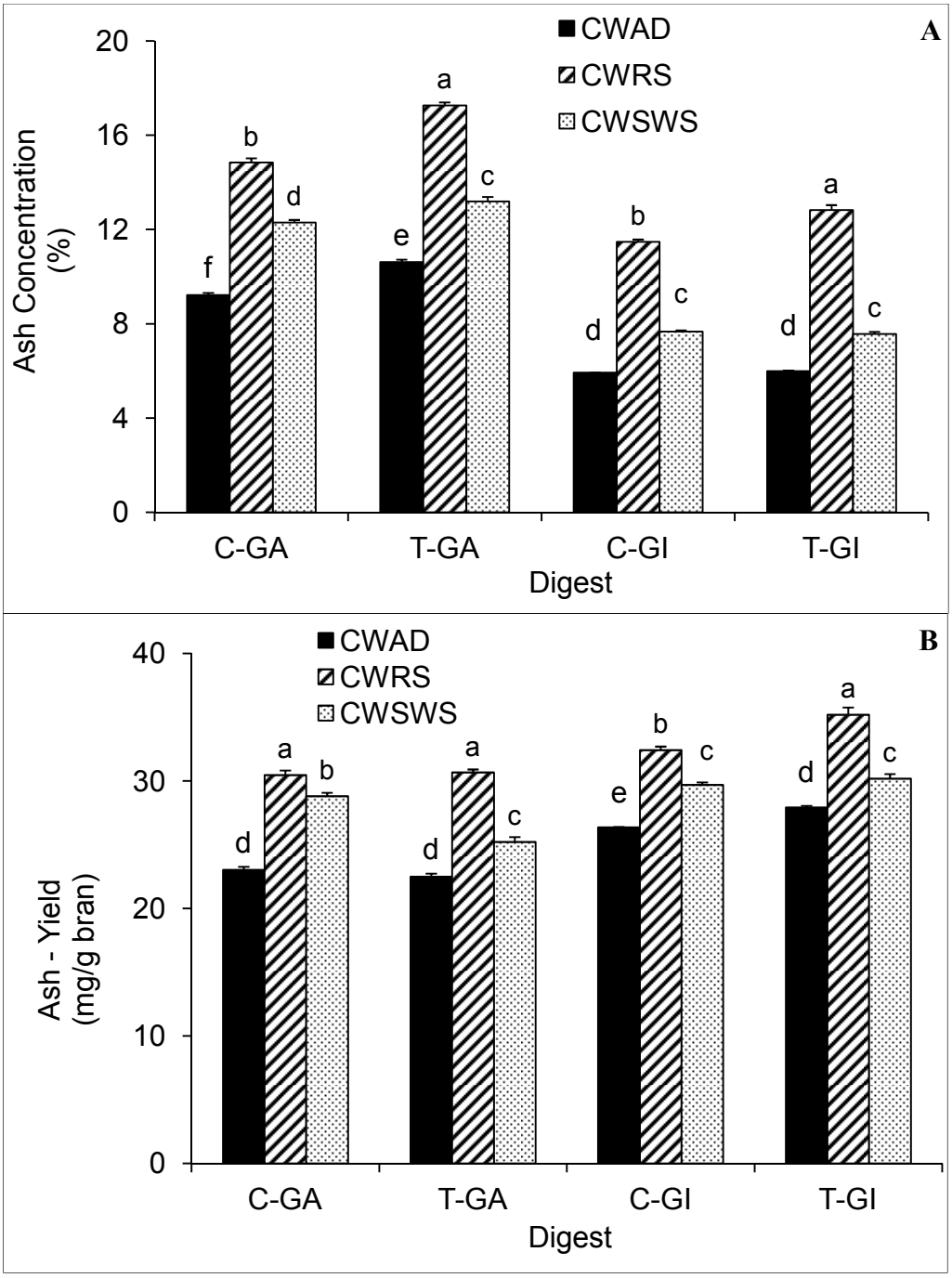


Figure 4.5. Ash concentration (A) and yield (B) in *in vitro* gastric (GA) and gastrointestinal (GI) digests of control (C) and autoclaved bran (T). Bars with different letters within each digest grouping are significantly different ($P < 0.05$)

Table 4.1. Bran moisture, ash, protein, and starch composition[a,b]

| Bran | Treat | Digest Constituents | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Moisture (%) | | Ash (%) | | Protein (%) | | Starch (%) | |
| | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | C | 8.81 | 0.22 | 5.46 b | 0.08 | 21.28 a | 0.34 | 21.99 b | 0.38 |
| | T | 7.48 | 0.12 | 5.61 b | 0.43 | 21.38 a | 0.05 | 24.52 a | 0.84 |
| CWRS | C | 9.00 | 0.48 | 7.73 a | 0.24 | 15.71 c | 0.05 | 5.74 d | 0.16 |
| | T | 7.47 | 0 | 7.78 a | 0.09 | 15.75 c | 0.10 | 5.44 d | 0.19 |
| CWSWS | C | 11.33 | 1.09 | 5.60 b | 0.11 | 18.75 b | 0.05 | 18.58 c | 0.84 |
| | T | 7.74 | 0.20 | 5.63 b | 0.00 | 18.95 b | 0 | 16.97 c | 1.15 |

[a] Different letters within columns are significantly different ($P < 0.05$)
[b] C, control; T, treated

4.3.1.2.1 Bioaccessibility of ash due to *in vitro* digestion of bran

The bioaccessibility of ash due to *in vitro* digestion of bran was substantial as it ranged from 39.4 to 53.6% (Table 4.2). Invariably, and as would be expected, the digestibility of ash due to combined effects of GA and GI treatments was higher than for GA digests alone. For control samples the average bioaccessibility of ash for GI digests was 47.8% compared to 44.4% for GA counterparts. For autoclaved bran digests the corresponding values were 49.5% (GI) vs. 41.4% (GA). For GA digests, autoclaving resulted in a significant decrease in ash digestibility for two of three brans (CWAD, CWSWS) (Table 4.2). That decrease was substantial for CWSWS bran (6.7%). For GI digests, there was an opposite effect; autoclaving resulted in a significant increase in ash digestibility for two of three brans (CWRS, CWAD). That increase was fairly substantial for the CWRS wheat bran (3.3%).

Table 4.2. Extractability of starch, protein and ash in *in vitro* digests of wheat bran[a,b]

| Sample | | | Extractability (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Starch | | Protein | | Ash | |
| | | | Mean | Stdev | Mean | Stdev | Mean | Stdev |
| CWRS | GA | Control | 1.75 b | 0.11 | 42.38 c | 0.25 | 39.4 c | 0.46 |
| CWRS | GA | Treated | 2.19 b | 0.06 | 23.19 d | 0.05 | 39.44 c | 0.31 |
| CWRS | GI | Control | 41.8 a | 0.90 | 52.64 a | 0.00 | 41.98 b | 0.36 |
| CWRS | GI | Treated | 41.1 a | 3.44 | 43.77 b | 0.26 | 45.3 a | 0.75 |
| CWAD | GA | Control | 0.84 c | 0.04 | 54.02 c | 0.17 | 42.2 c | 0.43 |
| CWAD | GA | Treated | 1.06 c | 0.00 | 34.37 d | 0.10 | 40.1 d | 0.41 |
| CWAD | GI | Control | 41.6 a | 1.22 | 72.21 a | 0.11 | 48.33 b | 0.03 |
| CWAD | GI | Treated | 37.8 b | 1.07 | 65.57 b | 0.00 | 49.74 a | 0.23 |
| CWSWS | GA | Control | 0.57 b | 0.05 | 50.25 c | 0.06 | 51.5 b | 0.49 |
| CWSWS | GA | Treated | 1.08 b | 0.10 | 29.77 d | 0.20 | 44.8 c | 0.68 |
| CWSWS | GI | Control | 38.8 a | 3.24 | 63.21 a | 0.10 | 53.05 a | 0.34 |
| CWSWS | GI | Treated | 44.8 a | 4.38 | 53.36 b | 0.11 | 53.6 a | 0.64 |

[a] GA, gastric; GI, gastrointestinal; Control, control bran; Treated, autoclaved bran.
[b] Columns within bran types with different letters are significantly different ($P < 0.05$).

### 4.3.2. Protein content of the three wheat brans

Protein concentration of CWAD, CWSWS, and CWRS wheat brans were 21.3, 18.7, and 15.7%, respectively (Table 4.1). Autoclaving had no effect on bran protein concentration (Table 4.1). Protein concentration varied significantly among the three wheat brans in the following order CWAD > CWSWS > CWRS. This ordering was the same as that found for starch concentration of the brans (Table 4.1).

#### 4.3.2.1 Protein content of CWRS bran digests and water extracts

Protein concentration of CWRS bran digests and water extracts ranged from 13.5% to 38.7% (Fig. 4.6A). The lowest concentration of protein occurred in simple

water extracts of autoclaved bran, and highest concentration was in GA and GI digests of control bran, and in GI digests of control bran prepared without pancreatin. Autoclaving of bran resulted in significantly lower protein concentration in corresponding samples of simple water extracts or *in vitro* GA and GI digests. Denaturation of protein that is likely to occur in the high temperature (121 $^{o}$C) autoclave conditions may have negatively affected their extractability.

Protein yields of CWRS bran digests and water extracts ranged from 12.3 to 82.7 mg/g bran. Comparing Figs. 4.6A and 4.6B, it can be seen that protein concentration and yield of water extracts and GA and GI digests of control and autoclaved CWRS wheat bran had similar patterns of variation. The GI digest of control bran had the highest protein yield (82.7 mg/g bran), while the 2.5 and 7.5 h water extracts of autoclaved bran were lowest in protein yield (12.3 and 13.2 mg /g bran, respectively) (Fig 4.6B). As might be expected, protein yields dropped significantly when GA and GI digests of CWRS wheat bran were carried out without pepsin and pancreatin enzymes, respectively. The decrease was much greater for the GA phase ( 66.6 vs. 36.7 mg/g bran) compared to the GI phase (82.7 vs. 71.8 mg/g bran). Interestingly, there was very little difference in protein content (concentration and yield) between simple water extracts of wheat bran (for 2 or 7.5 h) compared with water extracts of wheat bran carried out at pH 2 without pepsin. This result underscores the importance of pepsin for effective protein digestibility. The 2.5h and 7.5 h water extracts of control CWRS bran were both significantly higher in protein content (concentration and yield) than their corresponding autoclaved extracts. As noted previously for protein concentration

results, this outcome likely occurred due to reduced extractability of protein caused by high temperature autoclaving conditions.

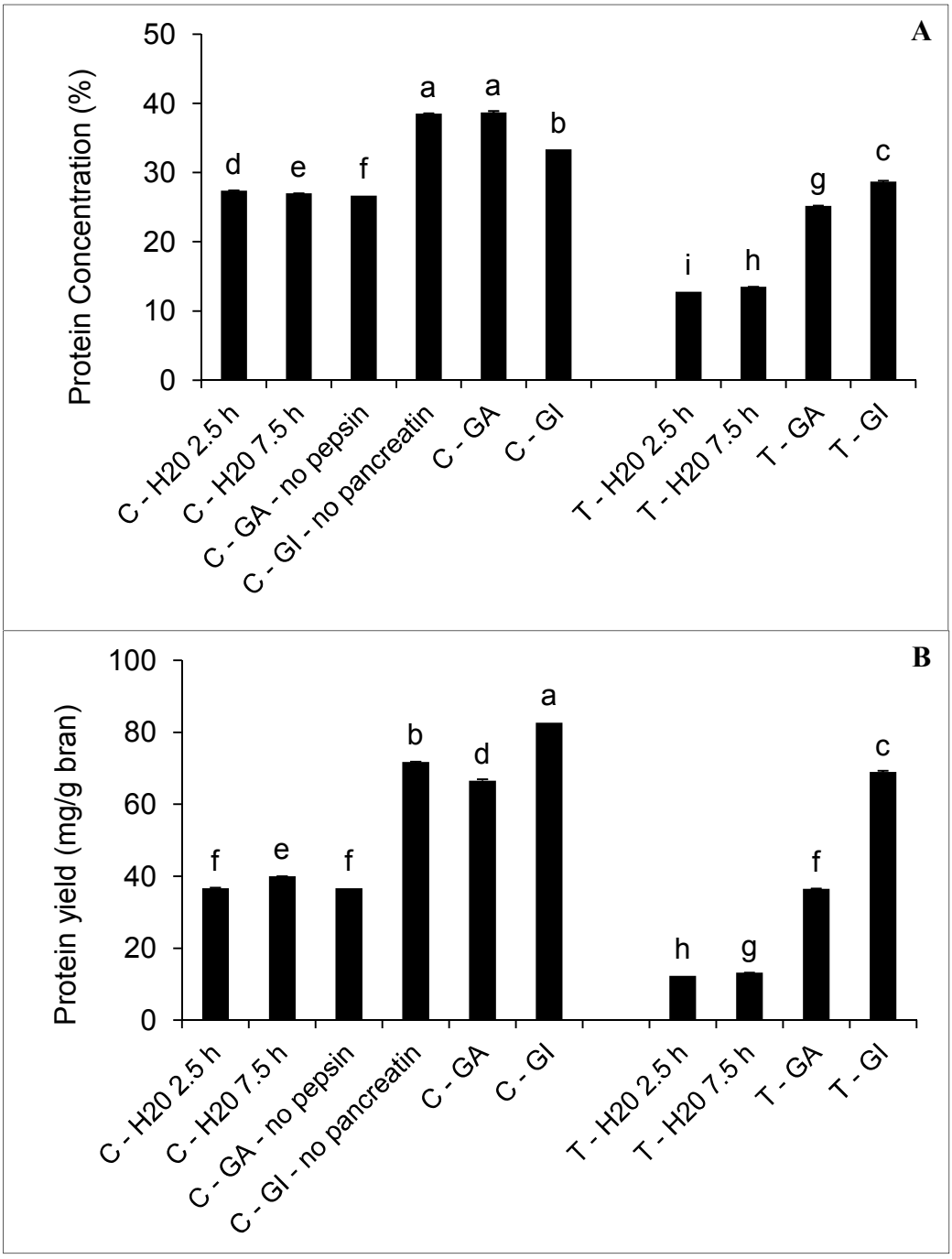


Figure 4.6. CWRS protein concentration (A) and yield (B) in *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, also 2.5 h and 7.5 h water extraction of control (C) and autoclaved bran (T). Bars with different letters are significantly different ($P < 0.05$).

4.3.2.2 Protein content of *in vitro* digests of the three wheat brans

Protein concentration of the three wheat bran digests ranged from 25.2% (GA of autoclaved bran) to 52.1% (GA of control bran) (Fig. 4.7A). The variation in protein yield of bran digests (Fig. 4.7B) within a given treatment group always followed the same order, i.e. CWAD > CWSWS > CWRS which corresponded to the pattern of variation noted above for bran protein. Yields of protein among digests ranged from 36.5 to 140.2 mg/g bran. As was observed for ash yields, GI digests always yielded significantly more protein than GA counterparts for a given bran type, however the increase was much greater than that found for ash content, i.e. 27.9% and 86.2% on average for control and autoclaved bran, respectively. In this case the incorporation of pancreatin, and constituent protease enzyme) in addition to the longer time frame of GI digestion most likely accounts for this increase. The considerably higher relative increase in protein yield for GI vs. GA digests of autoclaved bran compared to untreated bran suggests an interaction between autoclaving and *in vitro* digestion. However, that interaction appears to have its root in the fact that autoclaved bran had significantly lower protein yields than untreated brans, and those lower protein yields were relatively greater for GA digests compared to GI digests.

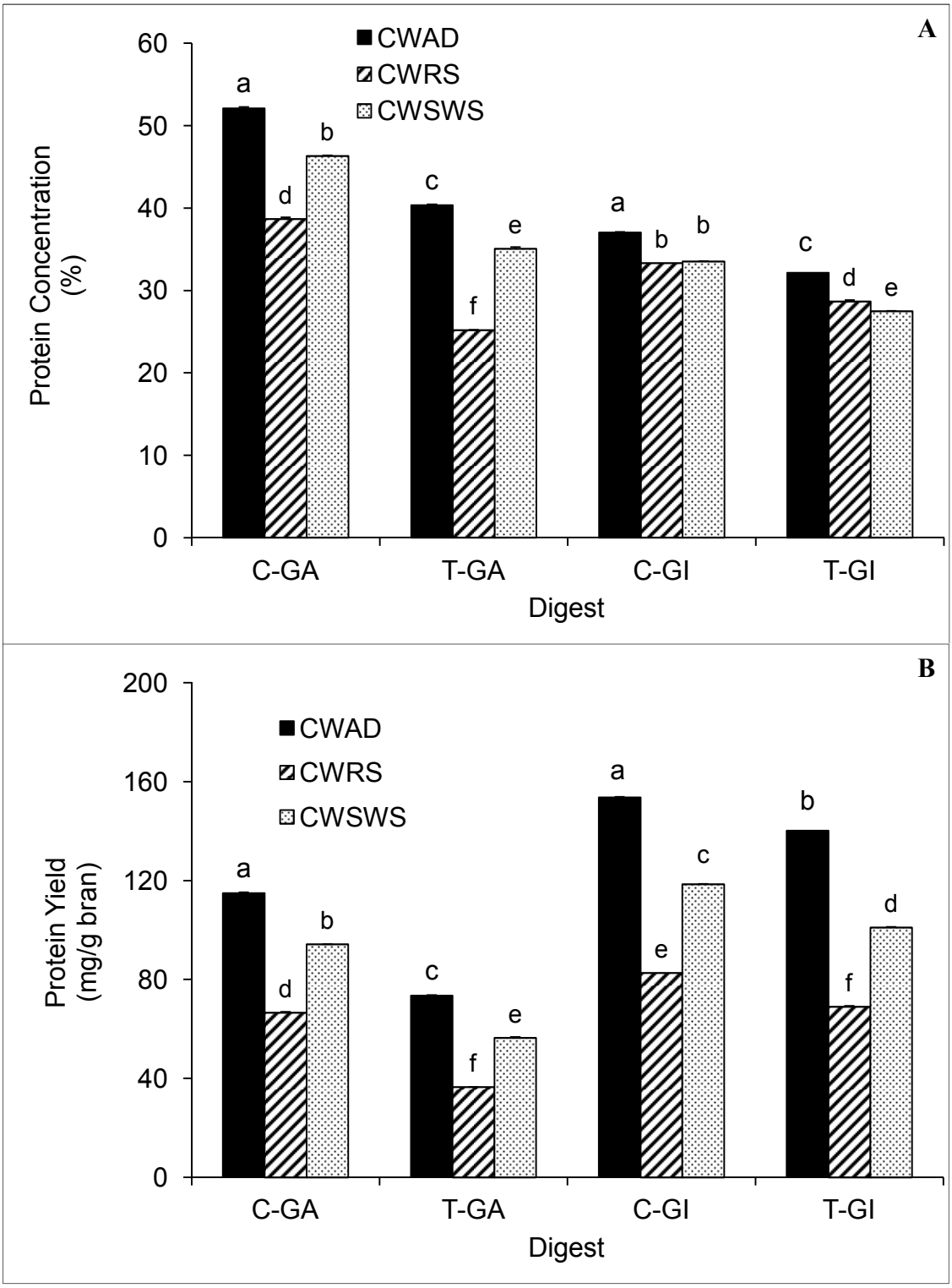


Figure 4.7. Protein concentration (A) and yield (B) in *in vitro* gastric (GA) and gastrointestinal (GI) digests of control (C) and autoclaved bran (T). Bars with different letters within each digest grouping are significantly different ($P < 0.05$).

4.3.2.2.1 Bioaccessibility of protein due to *in vitro* digestion of bran

The bioacessibility of protein due to *in vitro* digestion of bran was substantial as it ranged from 23.2 to 72.2% (Table 4.2) across all brans, digests and thermal treatments. Similar to ash digestibility results, and reflected in the above noted protein yield results, protein bioacessibility of all wheat bran digests increased after GI treatment compared to the GA *in vitro* treatment alone. For control samples the average bioaccessibility of protein for GI digests was 62.7% compared to 48.9% for GA counterparts. Protein digestibility appeared to vary considerably by bran type, and after GI digestion were 52.6%, 63.2%, and 72.2% for CWRS, CWSWS and CWAD control wheat brans, respectively. For autoclaved brans, the corresponding values were lower, i.e. 43.8%, 53.4% and 65.6% for CWRS, CWSWS and CWAD wheat brans, respectively. Accordingly, autoclaving of bran significantly decreased protein digestibility. This outcome may be due thermal denaturation of protein at the high autoclaving temperatures of 121 $^{o}$C which may have reduced the efficacy of protein hydrolysis during *in vitro* digestion.

4.3.3. Starch content of the three wheat brans

The three wheat brans varied widely in starch content. Starch concentration in control CWAD, CWSWS, and CWRS wheat brans were 22.0, 18.6, and 5.7%, respectively (Table.4.1). Bran starch content values for hard (CWRS) and soft (CWSWS) classes of common wheats are typical. The very high starch content for durum wheat is surprising given the long tempering time used of 72 h. It would appear

that the very hard kernel texture of durum wheat does not accommodate a satisfactory separation of bran from endosperm regardless of tempering time.

4.3.3.1 Starch content of CWRS bran digests and water extracts

While starch is referred to as such in this section of the thesis, its form as measured in GI digests (those with pancreatin, as discussed below) is most likely short chain dextrins due the presence of α-amylase enzyme in pancreatin.

Starch concentrations of *in vitro* digests of CWRS wheat bran and water extracts of that bran ranged from 0.6% to 9.7% (Fig. 4.8A) depending on the specific digest or sample. GI digests, containing pancreatin, of control and autoclaved bran had the highest starch concentrations. All other extracts or digests never exceeded 2.8% starch content.

The pattern of variation in starch content was very similar for both concentration and yield (Fig. 4.8). Starch yields ranged from 0.8 to 24.0 mg/g bran. CWRS wheat bran extracts containing the highest yields (Fig. 4.8B) were the GI digests of control and autoclaved brans which had similar high levels. All other digests had yields no higher than 3.7 mg/g bran. Results clearly indicated that presence or absence of pancreatin, and therefore α-amylase, in the GI digest was the determining factor in starch or dextrin yield, as digests prepared without pancreatin had starch yields < 2 mg/g for both control and autoclaved bran (Fig. 4.8B). Given the very low levels of starch in these digests or extracts (with most having non-significant differences) no discussion will be made.

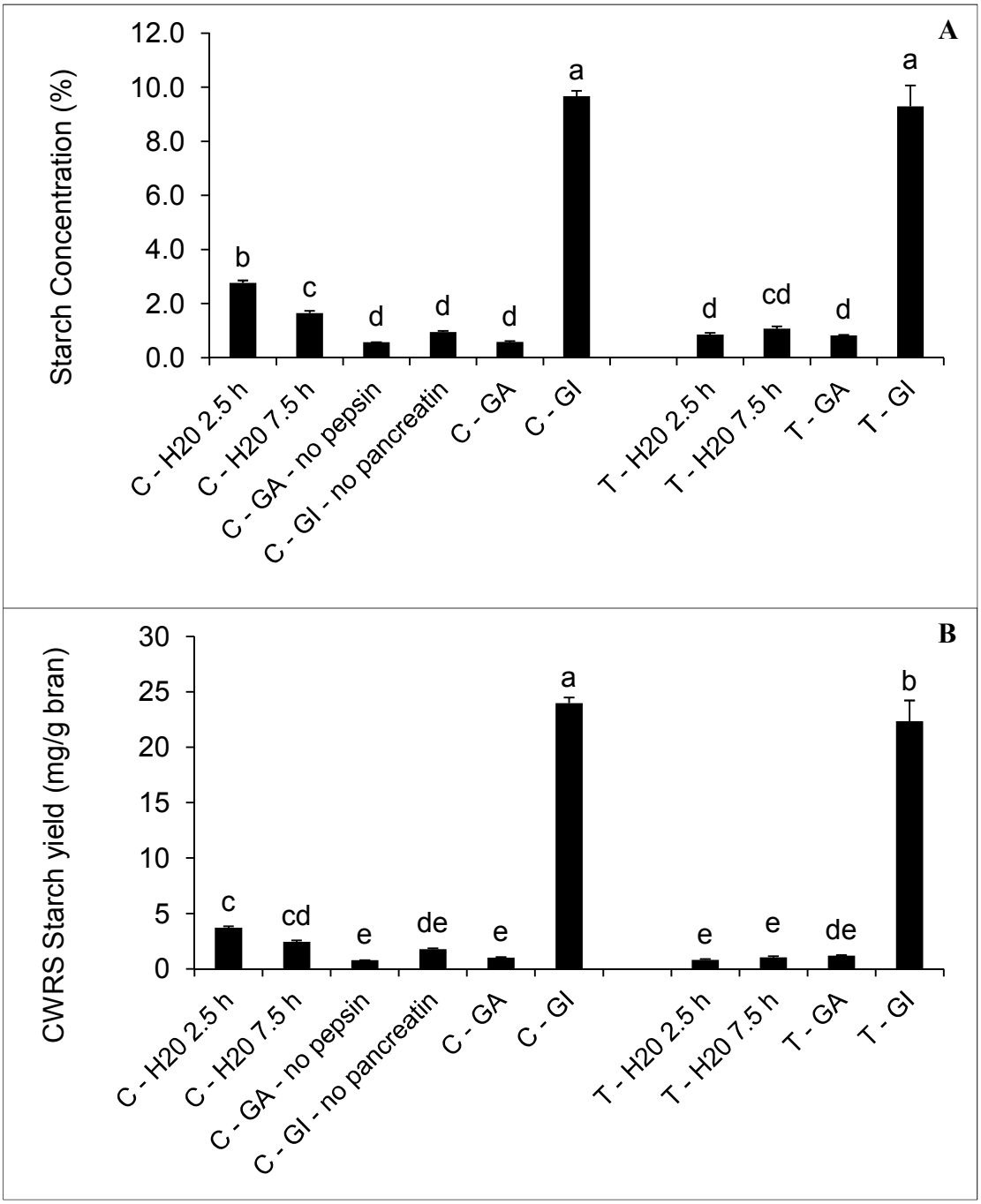


Figure 4.8. CWRS starch concentration (A) and yield (B) in *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, also 2.5 h and 7.5 h water extraction of control (C) and autoclaved bran (T). Bars with different letters are significantly different ($P < 0.05$).

4.3.3.2 Starch content of *in vitro* digests of the three wheat brans

The pattern of starch content (concentration and yield) variation of the *in vitro* digests of the three wheat brans was very similar (Fig. 4.9). Starch concentration ranged widely from 0.5% to 22.1% depending on bran and digest type. Autoclaving of bran had no significant or practical effect on starch content results of digests. As indicated in the previous section, significant levels of starch or dextrins in digests were only observed if the digest, i.e. the GI digest, contained pancreatin and α-amylase enzyme. For GI digests, starch content outcomes reflected the concentration of starch in the original brans. GI digests of CWRS wheat bran had substantially lower concentration and yield of starch compared to digests of CWSWS and CWAD bran. Yields of starch in GI digests of control bran were 24.0, 72.2, and 91.6 mg/g bran in CWRS, CWSWS and CWAD brans, respectively.

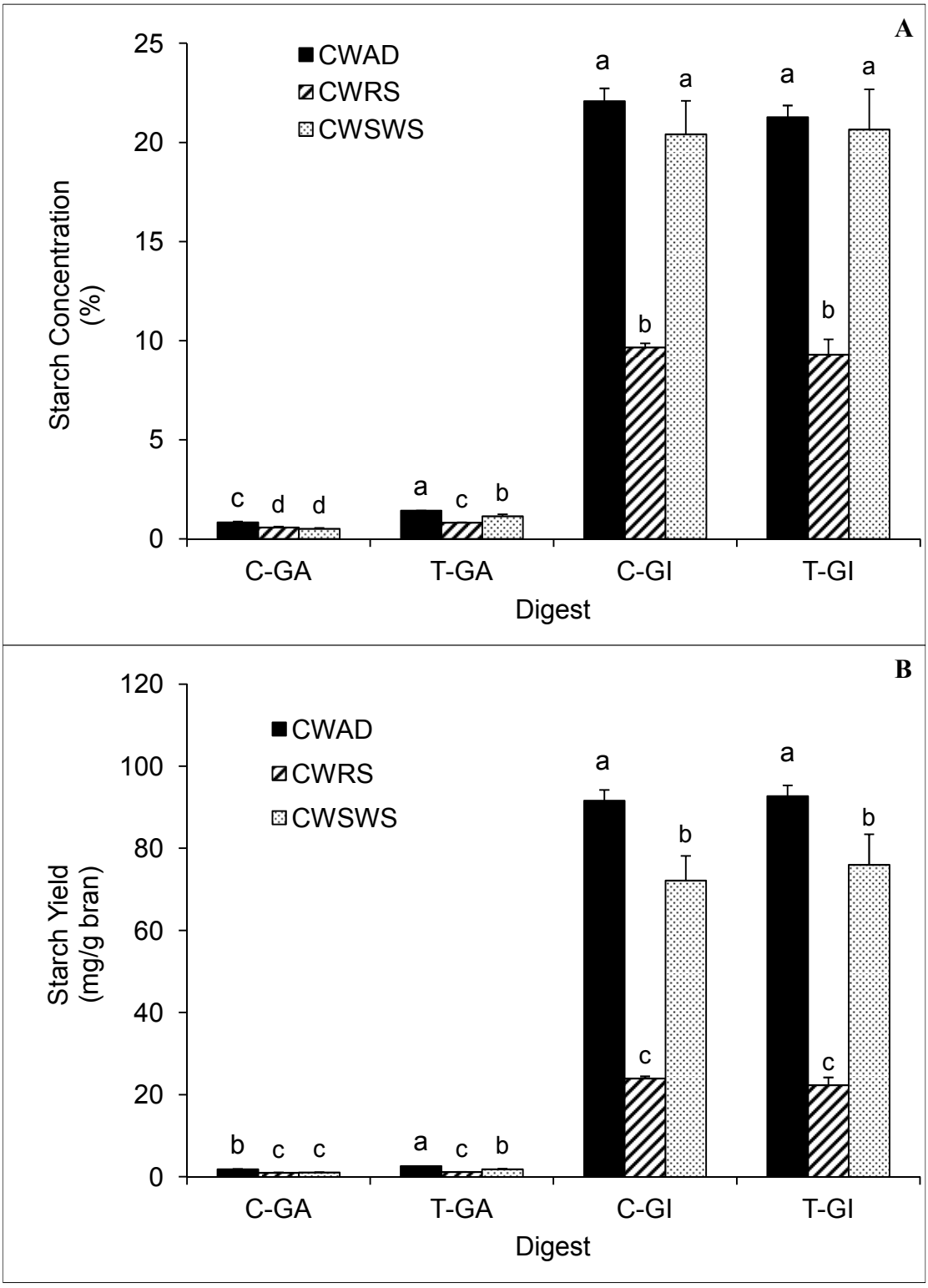


Figure 4.9. Starch concentration (A) and yield (B) in *in vitro* gastric (GA) and gastrointestinal (GI) digests of control (C) and autoclaved bran (T). Bars with different letters within each digest grouping are significantly different ($P < 0.05$).

4.3.3.2.1 Bioaccessibility of starch due to *in vitro* digestion of bran

The bioaccessibility of starch due to *in vitro* digestion of bran was substantial for GI digests only (Table 4.2). In spite of the large variation of starch content in the original brans, there was relatively very little variation in starch digestibility due to *in vitro* digestion which was measured as 41.8%, 38.8% and 41.6% for GI digests of CWRS, CWSWS and CWAD brans, respectively. GI digests of corresponding autoclaved bran had similar digestibilities of 41.1%, 44.8% and 37.8%, respectively. Accordingly, autoclaving had little to no effect on starch digestibilities after GI digestion. By comparison, digestibilities of starch after the GA phase of digestion were no higher than 2.2%, again most likely due to the absence of α-amylase in these digests.

4.3.4. Pentosan content of the three wheat brans

Concentration of pentosan in control CWAD, CWSWS, and CWRS wheat brans were 17.8, 22.9, and 28.3%, respectively (Table.4.3). Accordingly durum wheat bran had the lowest pentosan content, while CWRS wheat bran had the highest. No significant difference was found for pentosan content of brans due to autoclaving.

4.3.4.1 Pentosan content of CWRS bran digests and water extracts

Pentosan concentration of CWRS bran digests and water extracts ranged from 2.1 to 4.0 % (Fig. 4.10A). Among non-autoclaved, i.e. control, digests and extracts, there was no discernible pattern of variation; GA or GI digests were not different in

pentosan concentration compared to simple 2.5 h water extract, and the effect of added pepsin or pancreatin produced no clear outcome. It was unclear why the longer 7.5 h water extract of bran resulted in significantly lower pentosan levels compared to simple water extracts for 2.5 h. On the other hand, it was clear that autoclaving bran resulted in significantly higher concentrations of pentosans in simple water extracts as well as GA and GI digests. Those increases were 36.4% for 2.5 h water extracts, 55.8% for 7.5 h water extracts, and 52.8% and 35.8% for GA and GI digests respectively. Disruption or breakdown of cell wall fibre structure is the most likely reason for the increase in pentosan solubility arising from autoclaving of bran.

Yield of pentosans did not vary in a similar way to concentration results. The range of pentosan yield was from 3.1 to 8.4 mg/g bran. The CWRS bran extract with the highest pentosan yield was the GI-T digest and the lowest was the 7.5 h water extract of control bran, 7.5 h water extract of autoclaved bran, and GA-C-no pepsin digest. Pentosan yields of GA and GI digests of control bran were significantly higher than corresponding 2.5 and 7.5 h water extracts by 14.9 and 52.1%, respectively. Pentosan yield of the GA-C-no pepsin digest was significantly lower than its GA digest with pepsin. No similar effect was observed for the GI digest with respect to pancreatin addition. Pentosan yields of GA and GI digests of autoclaved bran were significantly higher than corresponding control digests by an average of 44.3%.

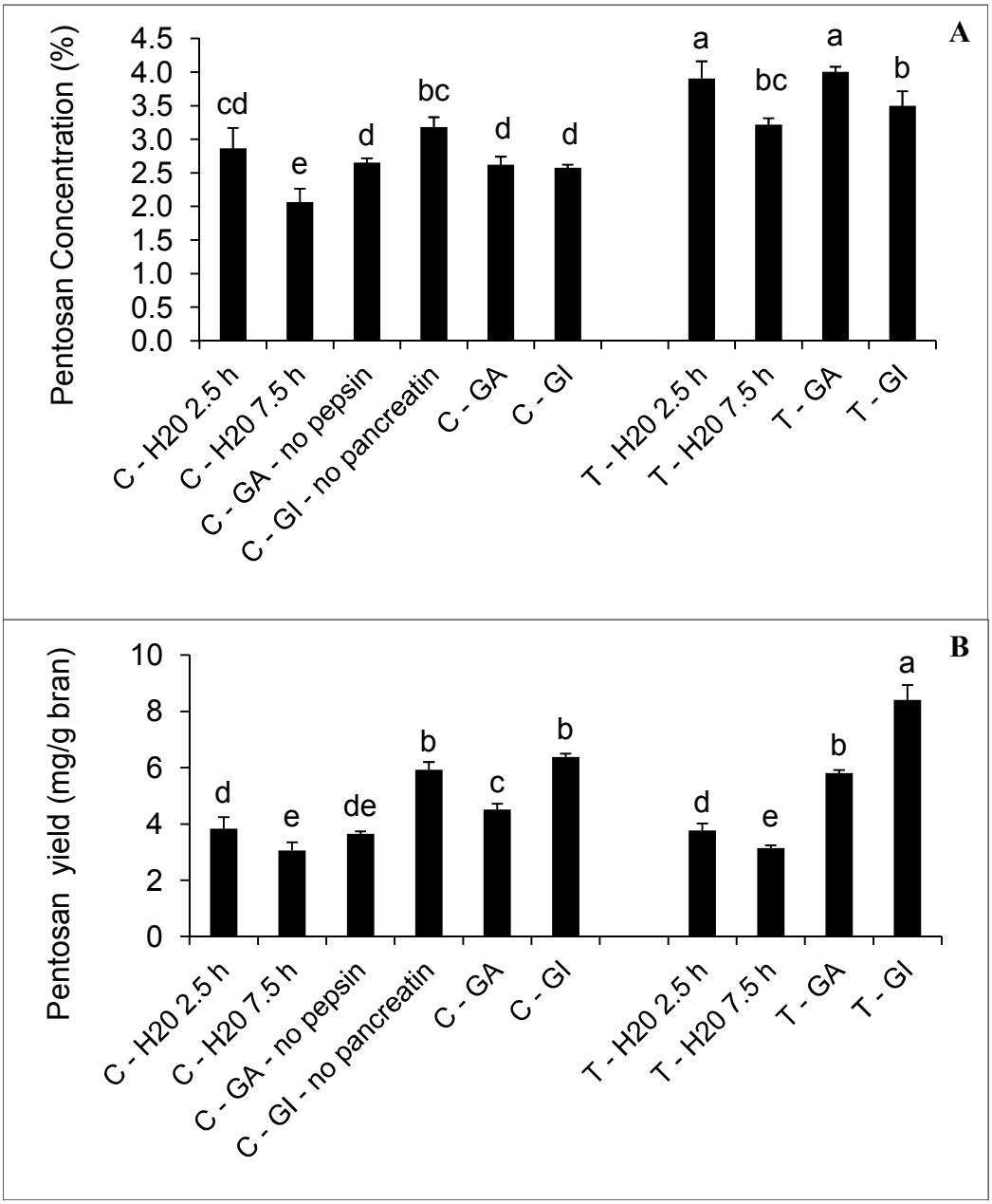


Figure 4.10. CWRS pentosan concentration (A) and yield (B) in *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, also 2.5 h and 7.5 h water extraction of control (C) and autoclaved bran (T). Bars with different letters are significantly different ($P < 0.05$).

4.3.4.2. Pentosan content of *in vitro* digests of the three wheat brans

Within each type of digest grouping (Fig. 4.11), durum wheat had the lowest pentosan concentration and yield, while the CWSWS bran digests (both GA and GI) had the highest pentosans yields. Pentosan concentration of the three wheat bran digests ranged from 0.5% to 22.1% (Fig. 4.11A). Yield of pentosans among digests ranged from 4.1 to 10.6 mg/g bran (Fig. 4.11B). Autoclaving of brans resulted in significant higher pentosan concentrations in GA digests for all brans and significantly higher penstosan concentrations in GI digests CWRS and CWSWS brans. For pentosan yield results, autoclaving of bran resulted in significantly higher values for CWRS bran GA digests only. For GI digests, autoclaving of all three wheat brans produced significantly higher pentosan yields (by 40.8 to 68.5%) compared to control bran.

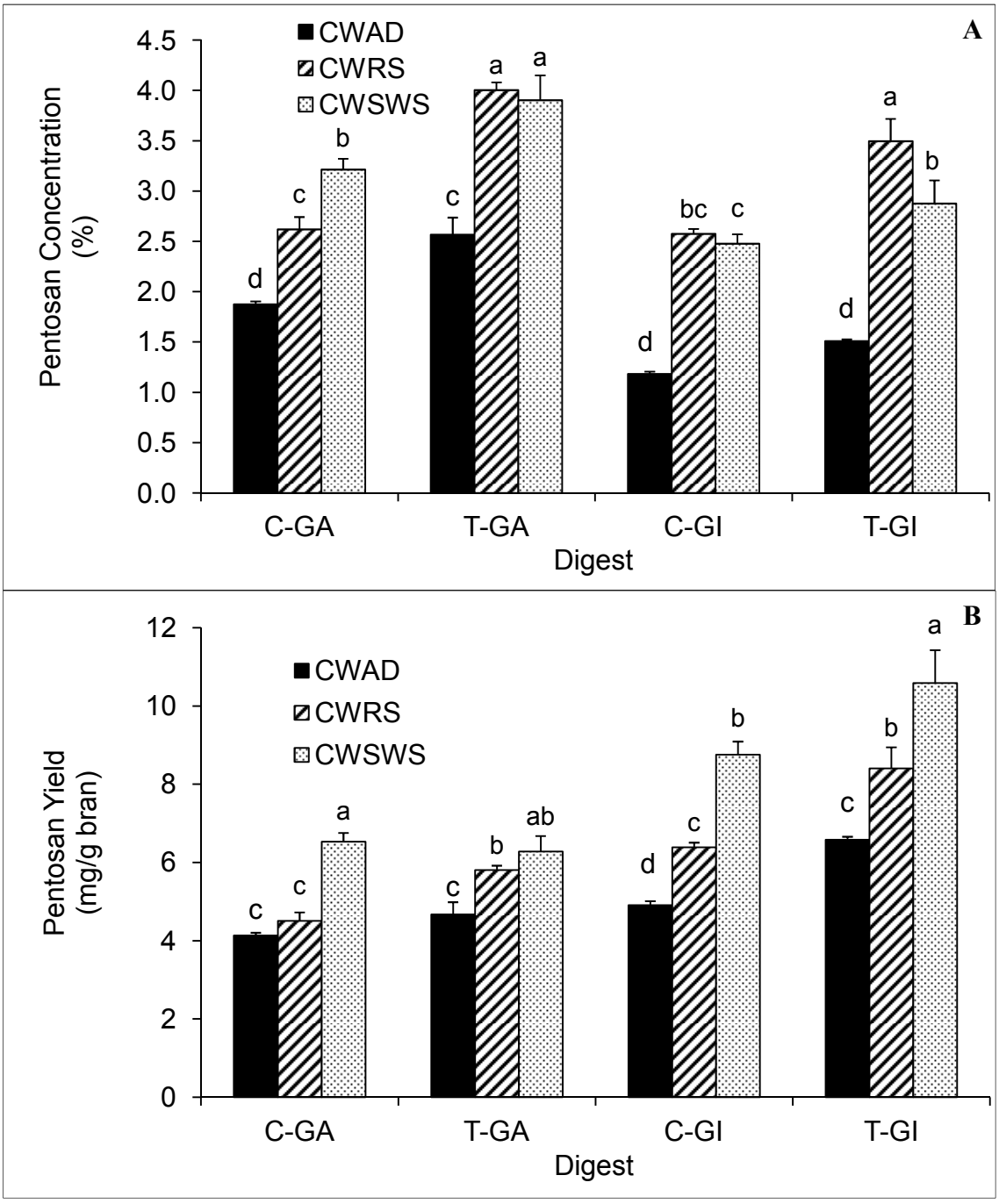


Figure 4.11. Pentosan concentration (A) and yield (B) in *in vitro* gastric (GA) and gastrointestinal (GI) digests of control (C) and autoclaved bran (T). Bars with different letters within each digest grouping are significantly different ($P < 0.05$).

Table 4.3. Bran pentosan, β-glucan, SDF, and IDF composition[a,b]

| Bran | Treat | Digest Constituents | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pentosan (%) | | β-glucan (%) | | SDF (%) | | IDF (%) | |
| | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | C | 17.76 c | 0.80 | 1.38 c | 0.01 | | | | |
| | T | | | 1.36 c | 0.01 | | | | |
| CWRS | C | 28.29 a | 0.18 | 2.60 a | 0.07 | 1.70 b | 0.15 | 52.94 a | 0.46 |
| | T | 27.65 a | 0.26 | 2.66 a | 0.14 | 4.91 a | 0.51 | 53.99 a | 0.10 |
| CWSWS | C | 22.90 b | 0.09 | 2.41 b | 0.07 | | | | |
| | T | | | 2.50 ab | 0.02 | | | | |

[a] Columns within bran types with different letters are significantly different ($P < 0.05$)
[b] C, control; T, treated; SDF, soluble dietary fibre; IDF, insoluble dietary fibre

4.3.4.2.1 Extractability of pentosan due to *in vitro* digestion of bran

The extractability of pentosan due to *in vitro* digestion of bran was much less than that observed for previously discussed bran constituents as it ranged from 1.6 to 4.6% (Table 4.4). The highly insoluble nature of bran fibre and the absence of any specific enzymes in the digests to degrade fibre are the most likely reasons for the low pentosan digestibilities. However, despite the low pentosan digestibilities and as with other constituents, the combined effect of GA and GI treatments was higher than for GA digests alone. For control samples the average extractability of pentosan for GA digests was 2.3% compared to 2.9% for GI counterparts. For autoclaved bran digests the corresponding digestibilities were 2.5% and 3.8% for GA and GI digests, respectively, indicating a positive effect of autoclaving for pentosan extractability which was significant for all bran types and digests with the exception of the CWSWS GA digest where no significant differenece was found. Autoclaving of CWRS wheat bran produced the most consistent increase in pentosan digestibility as relative increases were 32.1% and 34.5% for GA and GI digests, respectively. Corresponding values for GA

and GI digests of CWAD and CWSWS brans were 12.9% and 33.6%, and -3.8%% and 20.6%, respectively.

Table 4.4. Extractability of pentosan, β-glucan and fibre in *in vitro* digests of wheat bran[a,b]

| Sample | | | Extractability (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Pentosan | | β-glucan | | SDF | |
| | | | Mean | Stdev | Mean | Stdev | Mean | Stdev |
| CWRS | GA | Treated | 2.10 b | 0.04 | 11.09 b | 0.38 | 2.53 b | 0.13 |
| CWRS | GA | Control | 1.59 c | 0.07 | 11.12 b | 0.27 | 1.43 a | 0.11 |
| CWRS | GI | Treated | 3.04 a | 0.19 | 13.81 a | 0.00 | 2.55 b | 0.05 |
| CWRS | GI | Control | 2.26 b | 0.04 | 12.7 a | 0.85 | 1.51 a | 0.07 |
| CWAD | GA | Treated | 2.63 b | 0.17 | 13.13 b | 0.08 | | |
| CWAD | GA | Control | 2.33 c | 0.03 | 10.8 c | 0.90 | | |
| CWAD | GI | Treated | 3.70 a | 0.04 | 16.3 a | 0.41 | | |
| CWAD | GI | Control | 2.77 b | 0.05 | 12.37 b | 0.36 | | |
| CWSWS | GA | Treated | 2.74 c | 0.17 | 13.26 c | 0.00 | | |
| CWSWS | GA | Control | 2.85 c | 0.09 | 12.49 d | 0.03 | | |
| CWSWS | GI | Treated | 4.62 a | 0.37 | 18.70 a | 0.25 | | |
| CWSWS | GI | Control | 3.83 b | 0.14 | 15.6 b | 0.45 | | |

[a] GA, gastric; GI, gastrointestinal; Control, control bran; Treated, autoclaved bran; SDF, soluble dietary fibre

[b] Columns within bran types with different letters are significantly different ($P < 0.05$).

4.3.5. β-glucan content of the three wheat brans

Concentration of β-glucan in control CWAD, CWSWS, and CWRS control brans were 1.4, 2.4, and 2.6%, respectively (Table 4.3). No significant difference was found for β-glucan content of brans due to autoclaving. These low levels of β-glucan are typical for wheat and wheat bran which are lower than those for oats and barley (Henry, 1985).

4.3.5.1 β-glucan content of CWRS bran digests and water extracts

Concentration of β-glucan of CWRS bran digests and water extracts ranged from 0.9 to 3.1% (Fig. 4.12A) and followed a pattern somewhat similar to that for pentosan concentration (Fig. 4.10A) as among non-autoclaved extracts and digests there was no clear pattern. There was a slight but significant increase in β-glucan concentration in the control GA digest compared to the corresponding digest without pepsin. It was unclear why the longer 7.5 h water extract of control bran resulted in significantly lower levels (by 48.3%) of β-glucan compared to simple water extracts for 2.5 h control bran, however a similar trend was observed for pentosan concentration (Fig. 4.10A). As was observed for pentosan content, autoclaving of bran resulted in a very considerable increase in β-glucan concentration of water extracts, from 1.7% to 2.8% for 2.5 h extracts, and from 0.9% to 3.1% for 7.5 h water extracts. Autoclaving of bran also gave significant increases in β-glucan concentration of GA and GI digests, but those increases were not as large as for simple water extracts (Fig. 4.12A).

β-glucan yield results (Fig. 4.12B) varied in a more systematic manner. β-glucan yields of GA and GI digests of control bran were significantly higher than corresponding 2.5 and 7.5 h water extracts by 28.8 and 158.1%, respectively. GA and GI digests of bran without pepsin and pancreatin, respectively, were both significantly lower in β-glucan yield than their corresponding GA and GI digests with those enzymes. Also the 2.5 and 7.5 h water extracts of autoclaved bran were both significantly higher in β-glucan yield than their control bran counterparts. Autoclaving of bran resulted in a significant increase (by 22.9%) of β-glucan yield for GI digests. There wasn't a similar effect for GA digests of autoclaved bran.

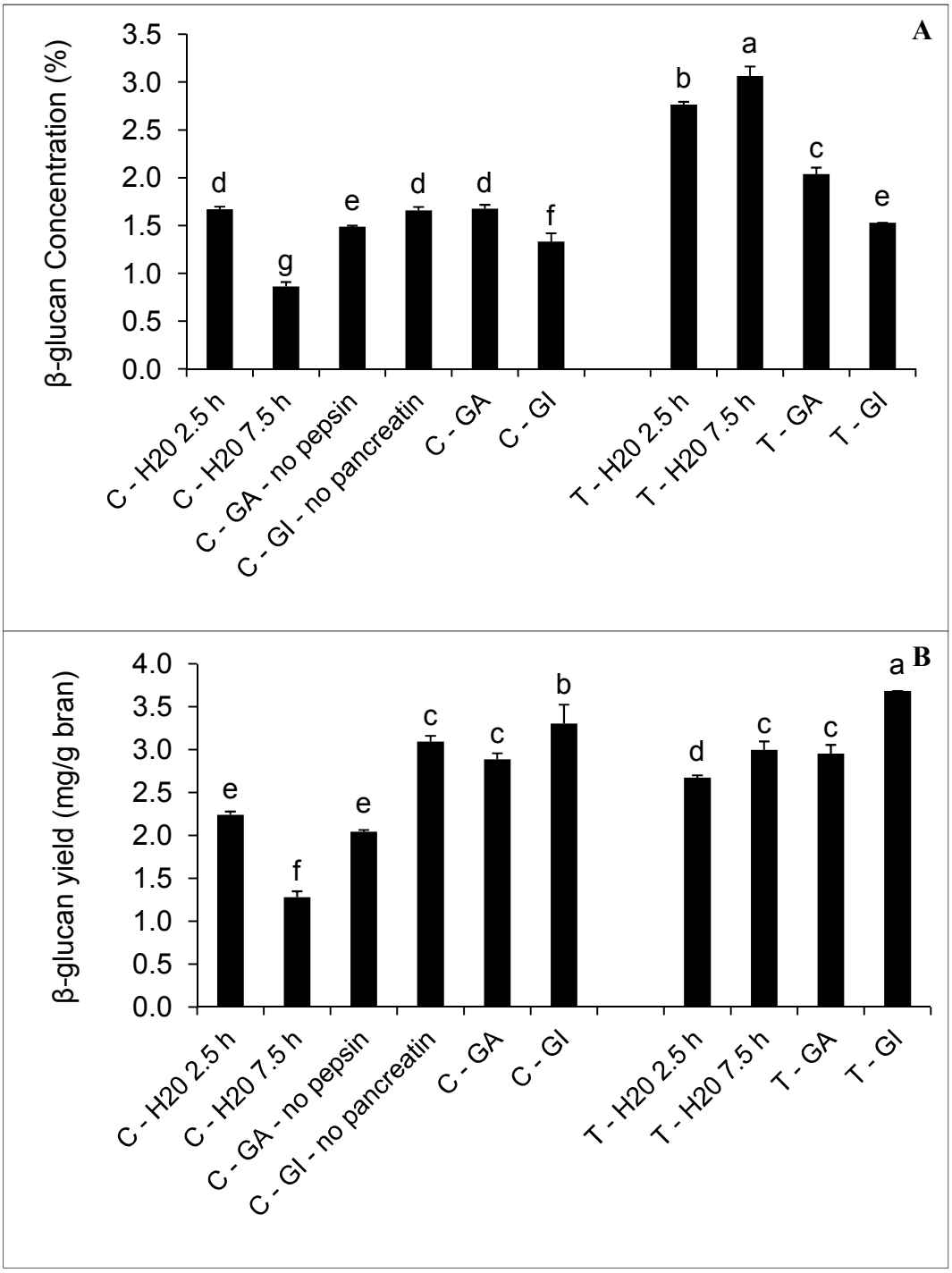


Figure 4.12. CWRS β-glucan concentration (A) and yield (B) in *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, also 2.5 h and 7.5 h water extraction of control (C) and autoclaved bran (T). Bars with different letters are significantly different ($P < 0.05$).

4.3.5.2. β-glucan content of *in vitro* digests of the three wheat brans

As with the levels determined for the three wheat brans used in this thesis research, β-glucan content (concentration and yield) of the three wheat bran digests were similarly low (Fig. 4.13) and followed the trend seen in the starting wheat brans with CWAD digests having the lowest values compared to digests of CWRS and CWSWS wheat bran. β-glucan in digest ranged in concentration from 0.4% to 2.1% (Fig. 4.13A) and in yield from 1.5 – 4.7 mg/g bran. β-glucan yield in GA digests of autoclaved bran was significantly higher than that of control bran of both CWAD (1.79 vs. 1.49 mg/g bran) and CWSWS (3.32 vs. 3.01 mg/g bran) brans not for CWRS bran digests. β-glucan yields in the GI digests all three wheat brans were significantly higher for autoclaved bran compared to control bran.

4.3.5.2.1 Extractability of β-glucan due to *in vitro* digestion of bran

The extractability of β-glucan due to *in vitro* digestion of bran ranged from 10.8 to 18.7% (Table 4.4) which reflected values much higher than those of pentosans (1.6 to 4.6%). The combined effects of the GA and GI treatments compared to the GA treatment alone produced a small increase in β-glucan extractability. For control samples, the average extractability of β-glucan for GA and GI digests was 11.5% and 13.5%, respectively. For autoclaved bran, the corresponding values were 12.5% and 16.3%. Accordingly, autoclaving of bran produced a significant increase in β-glucan extractability which occurred in two of three brans for both GA (CWRS and CWAD) and GI digests of all three brans.

4.3.6. Soluble dietary fibre content of *in vitro* digests of CWRS wheat bran

The concentration of soluble dietary fibre (SDF) of CWRS bran digests ranged from 3.1 to 9.9% (Fig. 4.14A). There was essentially no effect of pepsin or pancreatin addition in GA or GI digests, respectively. SDF concentrations of control GA and GI bran digests were 4.4% and 3.13%, respectively. In contrast SDF concentrations of GA and GI digests of autoclaved bran were on average were 102.8% higher at 9.9% and 5.7%, respectively. Similar results were found for SDF yields in digests of CWRS wheat bran (Fig.4.14B). Yields of SDF ranged from 6.2 to 14.4 mg/g bran. There was no effect of pancreatin addition for GI digests. However, SDF yield of CWRS bran GA digests was significantly reduced by the removal of pepsin. As with SDF concentrations, autoclaving of bran resulted in considerable increase in SDF yields of GA (7.6 vs. 14.4 mg/g) and GI (7.7 vs. 13.7 mg/g) digests. These SDF yields are ~ 25% higher when accounting for yields of other measured soluble fibre constituents, i.e. pentosans and β-glucan. This result is plausible as no doubt there are other soluble fibre components in minor amounts in bran digests that were not quantified, e.g. fructans. Haska et al. (2008) found 3 to 4 g/100 g of fructans in brans of three different wheats.

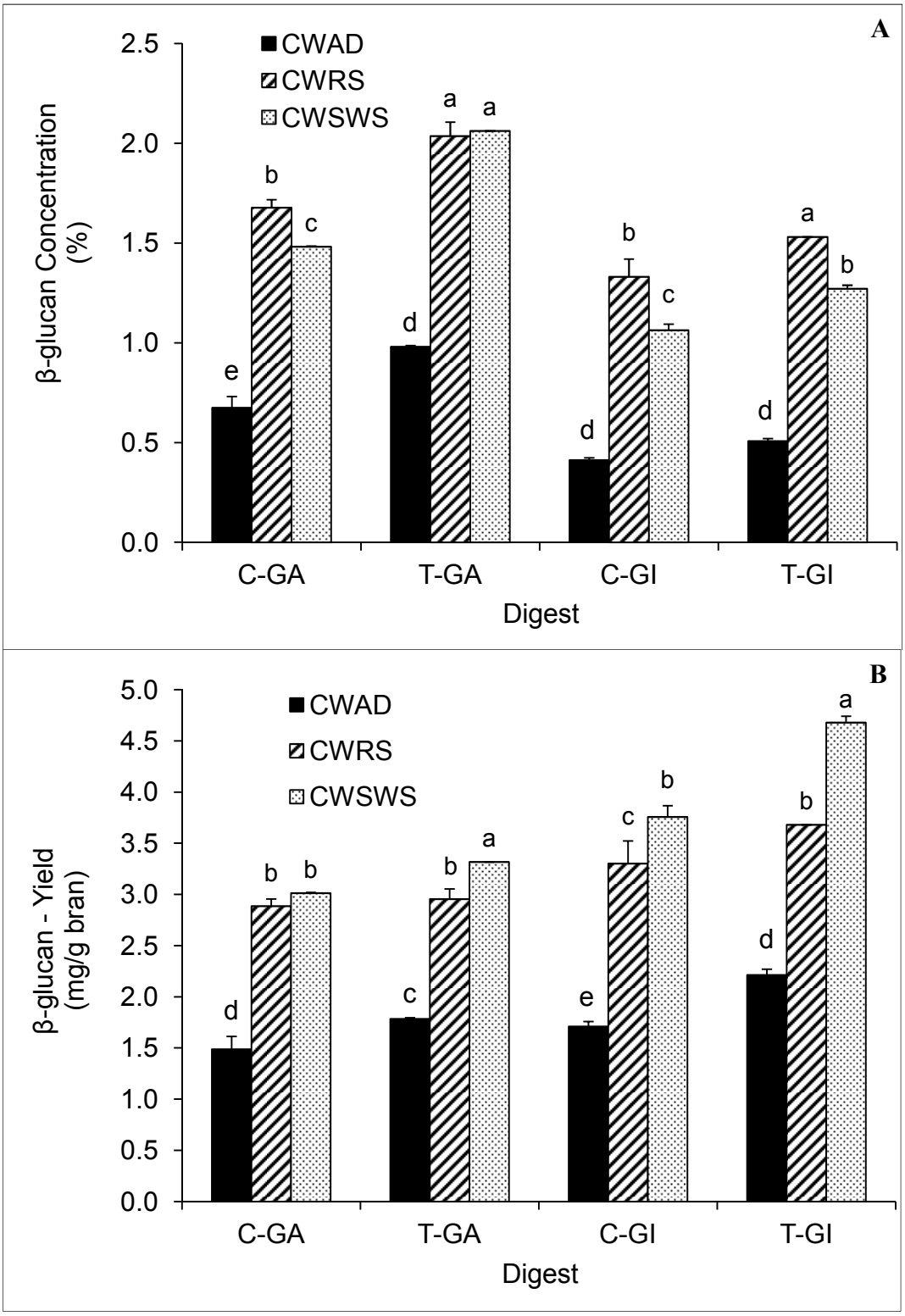


Figure 4.13. β-glucan concentration (A) and yield (B) in *in vitro* gastric (GA) and gastrointestinal (GI) digests of control (C) and autoclaved bran (T). Bars with different letters within each digest grouping are significantly different ($P < 0.05$).

4.3.6.1. Extractability of SDF due to *in vitro* digestion of bran

The extractability of SDF due to *in vitro* digestion of bran was low and comparable in absolute amount to that of pentosans (Fig. 4.4). SDF extractability ranged from 1.4 to 2.5%. Autoclaved bran digests were 68.9% and 76.9% higher in corresponding GA and GI digests, respectively. In contrast to digest outcomes for other specific fibre components, pentosans and β- glucan, SDF extractability was significantly lower in GI digests compared to GA counterparts. An explanation for this results in unclear.

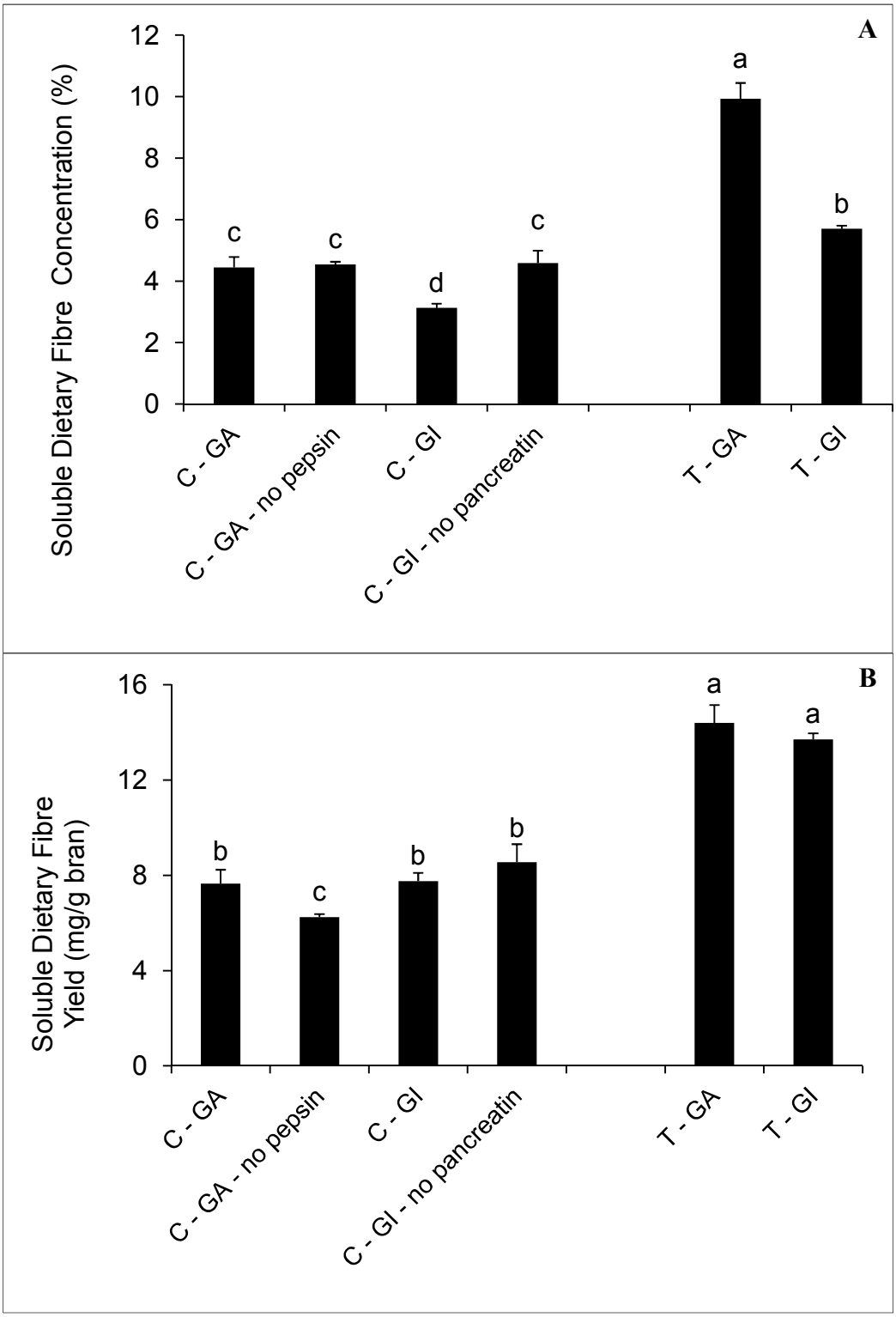


Figure 4.14. CWRS soluble dietary fibre (SDF) concentration (A) and yield (B) in *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, also 2.5 h and 7.5 h water extraction of control (C) and autoclaved bran (T). Bars with different letters are significantly different ($P < 0.05$).

### 4.3.7. DPPH radical scavenging activity

#### 4.3.7.1 DPPH radical scavenging activity content of CWRS bran digests and water extracts

DPPH radical scavenging activity concentration of CWRS bran digests and water extracts ranged from 21.9 to 166.1 μmol Trolox Equivalents (TE)/g (Fig. 4.15). Within control and autoclaved groups of bran digests or extracts, water extracts had substantially and significantly lower concentrations (Fig. 15A) as well as the lower yields (Fig. 15B) of radical scavenging activity. Accordingly, simply acidifying water extraction conditions (i.e. GA-no pepsin results) resulted in very considerable increases in free radical scavenging activity of those extracts. For example there was a 5.0X increase in yield of free radical scavenging activity for the GA-no pepsin treatment on control bran (used 2.5 h time frame) compared to the 2.5 h water extract of control bran. For the GI-no pancreatin result which was a 7.5 h digest of control bran, the corresponding difference in yield of free radical scavenging activity was 3.3X greater than the corresponding 7.5 h water extract of control bran. For yield results, the addition of pepsin and pancreatin in the GA and GI digests, further increased DPPH radical scavenging by 46.6% and 23.9%, respectively.

Autoclaving of bran significantly increased DPPH scavenging activities (concentrations or yields) only for water extracts of bran, with the exception of GA digests (concentrations) which had higher free radical scavenging activity (by 11.3%) compared to the GA digest of the untreated control bran.

4.3.7.2 DPPH radical scavenging activity of *in vitro* digests of three wheat brans

Concentration of DPPH radical scavenging activity in GA and GI digests of the three wheat brans ranged from 85.1 to 154.6 µmol TE/g (Fig. 4.16A). The variation appeared to be mainly attributable to type of bran, as CWRS wheat bran digests invariably had significantly higher concentration of free radical scavenging activity compared to digests of the other two brans. DPPH radical yield results (Fig. 4.16B) also revealed some significant differences among bran types, but there was no clear pattern. Yields of DPPH radical scavenging activity per g bran across digests ranged from 18.2 to 47.2 µmol TE. GI digests had considerably higher yields (average of 62.1%) of DPPH radical scavenging activities on average compared to GA digests. Autoclaving of bran significantly affected the yield of free radical scavenging activities of the CWAD bran where DPPH radical scavenging activity of the GA and GI digests increased from 23.2 µmol to 26.7 µmol TE/g bran and from 37.3 to 47.2 µmol TE/g bran, respectively.

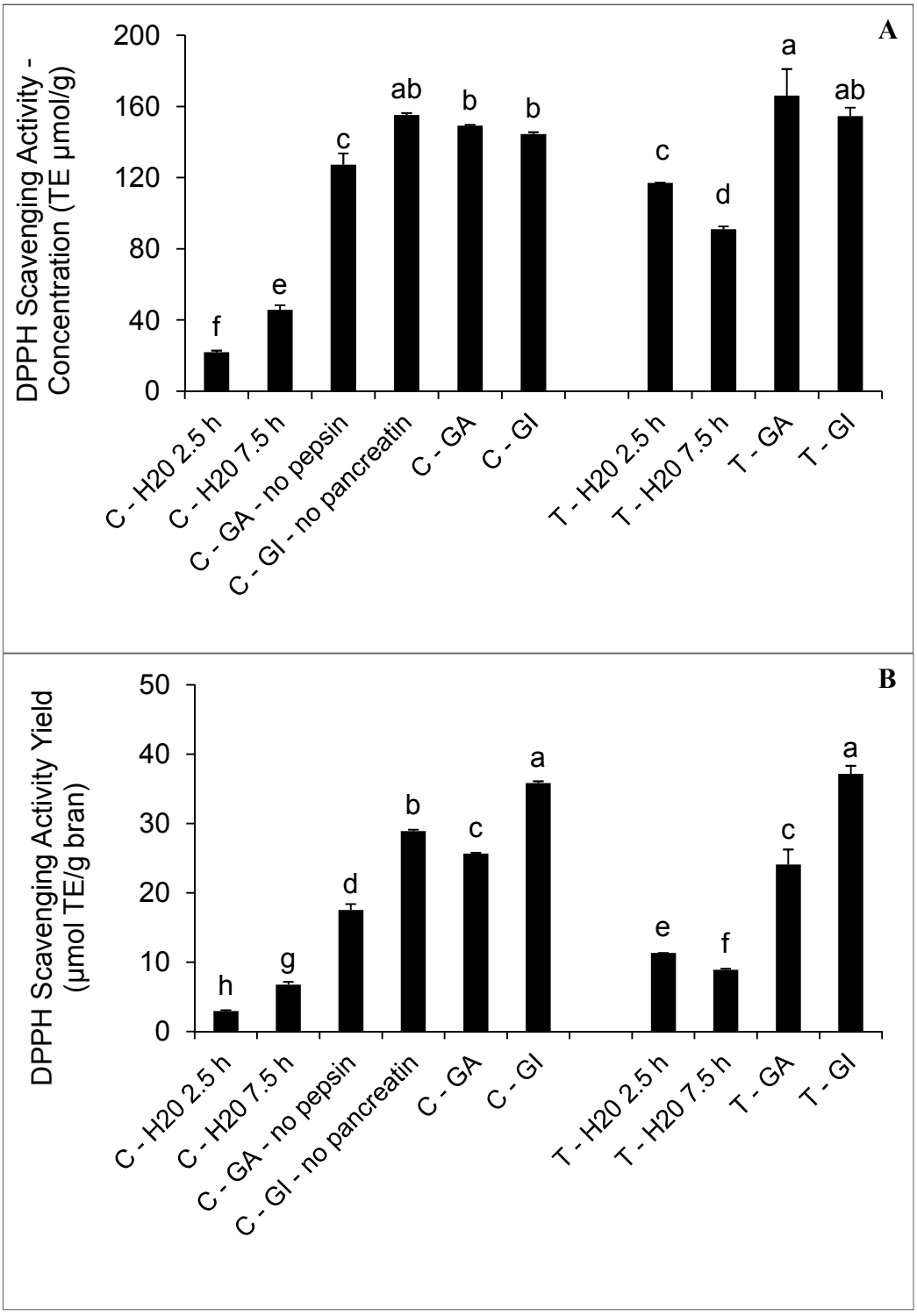


Figure 4.15. CWRS DPPH scavenging activity concentration (A) and yield (B) in *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, also 2.5 h and 7.5 h water extraction of control (C) and autoclaved bran (T). Bars with different letters are significantly different ($P < 0.05$).

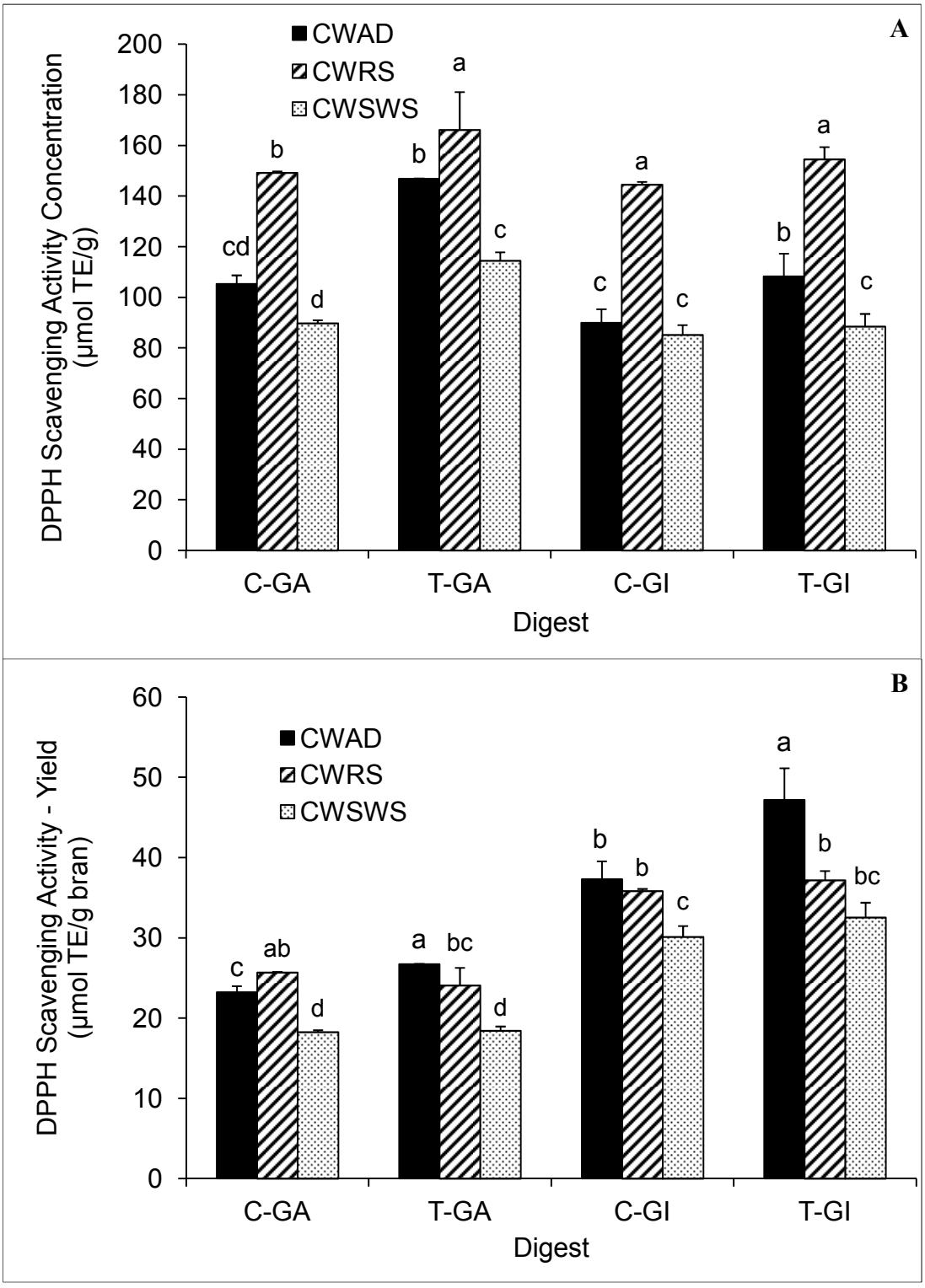


Figure 4.16. DPPH scavenging activity concentration (A) and yield (B) in *in vitro* gastric (GA) and gastrointestinal (GI) digests of control (C) and autoclaved bran (T). Bars with different letters within each digest grouping are significantly different ($P < 0.05$).

4.3.8. Metal chelating activity content

4.3.8.1 Metal chelating activity content of CWRS bran digests and water extracts

Metal chelation activity provided another measure of antioxidant activity of digests and extracts. The concentration of metal chelating activity of CWRS bran digests and water extracts (Fig. 4.17A) followed a somewhat similar pattern of variation compared to DPPH radical scavenging activity (Fig. 4.15A). Results ranged from 35.9 to 154.4 μmol $Na_2EDTA$ equivalents/g bran (Fig. 4.17A). Water extracts within a treatment type (i.e. autoclaved or not) had the lowest concentration (and yield) of metal chelating activity. As was observed for DPPH scavenging activity, simply acidifying water extraction conditions (i.e. GA-no pepsin results) resulted in considerable increase in metal chelation activity of those extracts (compare C-H2O 2.5 h vs. C-GA no pepsin). Likewise, there was a 3.0X increase in concentration of metal chelating activity for the GI-no pancreatin result (7.5 h digest) compared to the water extract with the same 7.5 h extraction time. On the other hand, GA and GI digests carried out without pepsin and pancreatin, respectively, had no effect on concentration of metal chelating activity of the same digests with pepsin and pancreatin (Fig. 4.17A). Autoclaving of bran resulted in significantly increase concentration of metal chelation activity of water extracts.

GI digests (whether for control or autoclaved bran) had the highest yield of metal chelating activity, which for the GI-C digest was 5.5X higher than the 7.5 h water extract of control bran (lowest yield) (Fig. 4.17B). Water extracts in general had the lowest yields of metal chelation activity. *In vitro* GA and GI digests of control bran had significantly higher yields of metal chelation activity compared to corresponding

digests, i.e. GA-no pepsin and GI-no pancreatin. Autoclaving of bran had only a small effect on increasing yield of metal chelating activity which was significant only for the 7.5 h water extract.

4.3.8.2. Metal chelating activity of *in vitro* digests of three wheat brans

Concentration of metal chelating activity of the three wheat bran digests ranged from 87.8 to 154.4 μmol $Na_2EDTA$ equivalents/g (Fig.4.18A). CWRS bran had the highest concentration of metal chelation activity of all digest types, while CWAD bran had the lowest in GA and GI digests of control bran only. Autoclaving of bran had no effect on the concentration of metal chelation activity in digests

Yield of metal chelating activity across digests ranged from 21.5 to 44.0 μmol$Na_2EDTA$ equivalents/g bran. As was observed for yield of DPPH radical scavenging activity (Fig. 4.16B), GI digests had noticeably higher yields of metal chelation activity by an average of 60.6% compared to GA counterparts. Within GA digests, there were no significant differences in metal chelating activity among bran types. For GI digests of autoclaved bran, CWAD and CWSWS brans had significantly higher metal chelation activity compared to CWRS bran. Autoclaving of bran had little effect yield on metal chelating activity of bran digests. For CWAD and CWSWS wheat bran, autoclaving reduced the yields of metal chelation activity in GA digests by 12.8% and 19.2%, respectively. For CWAD wheat bran, autoclaving of bran increased yield of metal chelation activity in GI digests by 20.8% from 36.4 to 44.0 μmol $Na_2EDTA$equivalents/g bran.

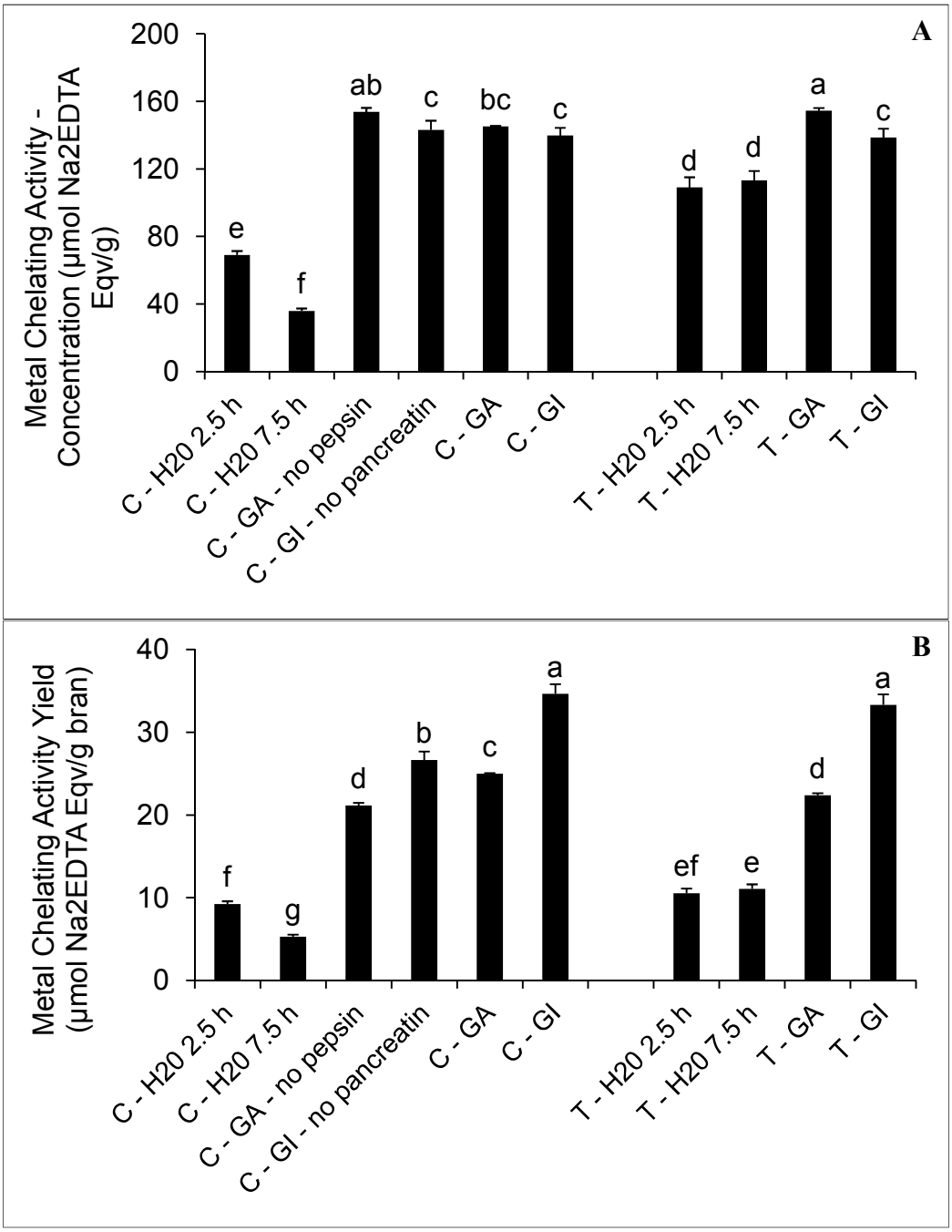


Figure 4.17. CWRS metal chelating activity concentration (A) and yield (B) in *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, also 2.5 h and 7.5 h water extraction of control (C) and autoclaved bran (T). Bars with different letters are significantly different ($P < 0.05$).

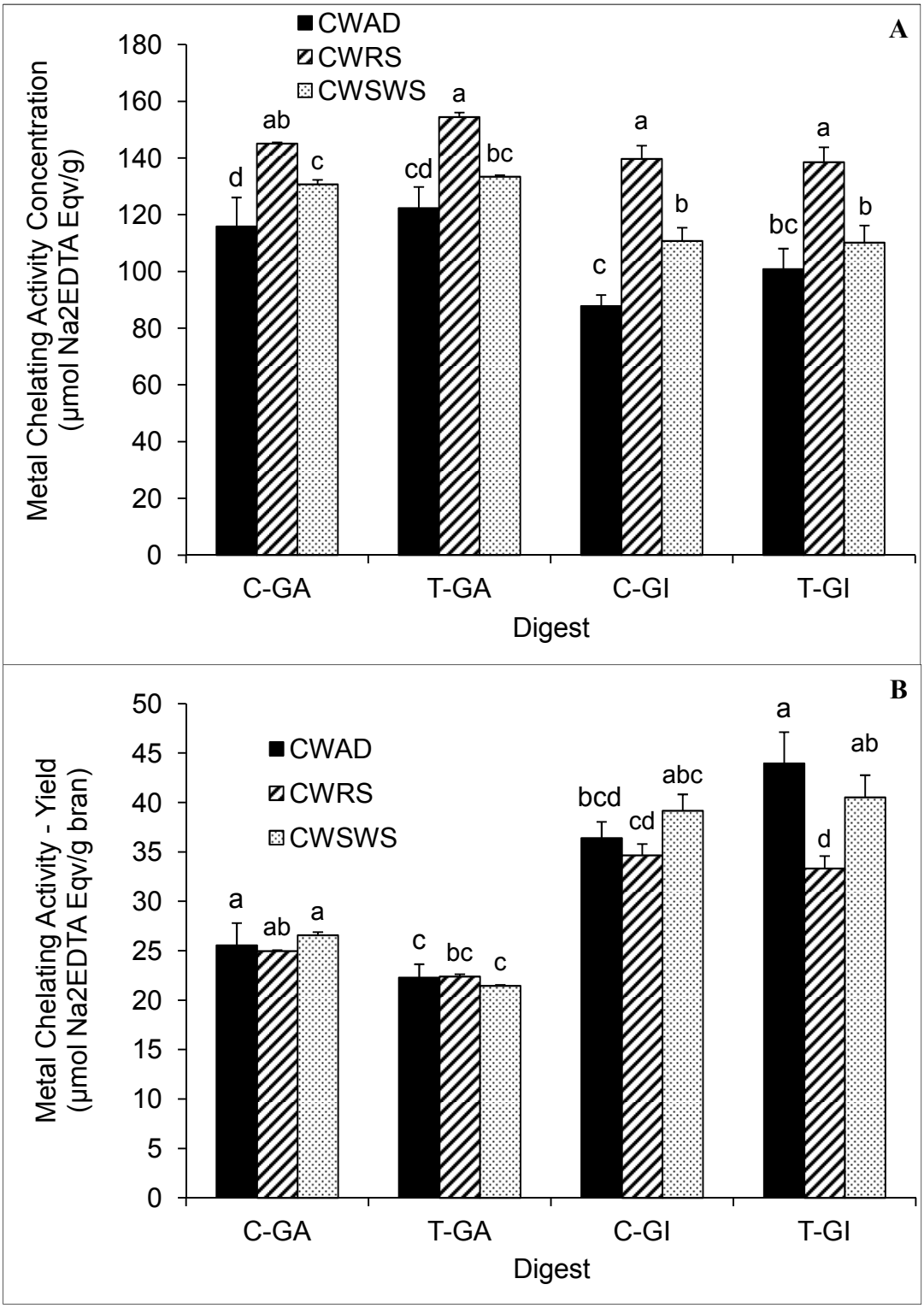


Figure 4.18. Metal chelating activity concentration (A) and yield (B) in *in vitro* gastric (GA) and gastrointestinal (GI) digests of control (C) and autoclaved bran (T). Bars with different letters within each digest grouping are significantly different ($P < 0.05$).

### 4.3.9. Total phenolic content

#### 4.3.9.1 Total phenolic content of CWRS bran digests and water extracts

Total phenolic content (TPC) provided a third complementary measure of antioxidant activity in this thesis research, and results followed in general similar trends to those found for DPPH radical scavenging activity and metal chelation activity (Figs. 4.21 & 4.22).

Concentration of TPC of CWRS wheat bran digests and water extracts ranged from 33.0 to 76.9 μmol ferulic acid equivalents (FAE)/g (Fig. 4.19A). GI digests had the highest concentrations, as well as yields (Fig. 4.19B) of TPC, indicating that more extensive digestion, as accommodated by the GI treatment in terms of time and enzymes, was necessary to maximally extract phenolic compounds from wheat bran. Simple water extracts of control non-autoclaved wheat bran had lower concentration levels of TPC compared to normal GA and GI digests. The lowest concentration sample was the 2.5 h water extract. As well, the inclusion of pepsin and pancreatin in GA and GI digests, respectively, of control wheat bran, significantly increased concentration of TPC by moderate amounts (Fig. 4.19A). Autoclaving of bran did not result in an increase in concentration of TPC in digests.

Yield results for TPC (Fig. 4.19B) followed a pattern very similar to that of TPC concentration in extracts and digests of CWRS wheat bran. Most noteworthy was the substantial increase of yield of TPC in GI digests, whether from autoclaved bran or not, compared to GA digests or water extracts. As was observed for the concentration results, simple water extracts had the lowest yield of TPC, and the use of pepsin and

pancreatin in GA and GI digests, respectively, of control wheat bran, significantly increased yield of TPC by 77.2 and 82.5%, respectively (Fig. 4.19B).

4.3.9.2. Total phenolic content of *in vitro* digests of three wheat brans

Concentration of TPC in digests of the three wheat brans ranged from 38.9 to 76.9 µmol FAE/g (Fig. 4.20A) and followed a pattern of variation similar to that observed for DPPH radical scavenging activity and metal chelation activity of digests, i.e. CWRS wheat bran was associated with the highest concentrations of TPC for a given digest type, and autoclaving of bran did not result in an increased concentration of TPC in digests.

Yields of TPC per g of bran across digests ranged almost five-fold, from 5.1 to 24.1 µmol FAE/g bran (Fig.4.20.B), which is equivalent to 1.0 to 4.7 mg FAE/g bran. As was observed for DPPH radical scavenging activity and metal chelation activity of digests, CWAD bran was associated with the highest TPC yields which were found in the GI digests. Similarly, GI digests had considerably higher yield, by an average of 171.4%, of TPC compared to GA digests.

Also as was observed for metal chelation activity, the effect of bran autoclaving resulted in a significantly decreased yield of TPC for GA digests on average by 26.6% on average. For GI digests autoclaving of bran likewise significantly reduced yield of TPC on average by 13.6% on average. It is not clear why autoclaving of bran caused a decline in TPC yield *of in vitro* digests. There are known issues associated with the Folin-Ciocalteu (F-C) method that may contribute to inaccurate measurement of true

phenolic content. These inaccuracies possibly relate to the non-specific nature of the F-C reaction that can lead to underestimating (not common, Singleton et al. 2009) as well as overestimating phenolic compounds, as the reaction with the F-C reagent in alkaline can quantify oxidizable non-phenolic compounds that are co-extracted with true phenolics and are capable of reducing the F-C reagent complex, such as ascorbic acid, sulfites, aromatic amino acids tyrosine and tryptophan, and cysteine (Singleton et al. 2009). The latter amino acids can be expected to be found in proteins of wheat bran, and it was previously observed that autoclaving reduced protein yield in digests (Fig. 4.8). However, the nature of the extraction of the digests for the F-C method with 100% methanol should have been sufficient to eliminate any protein from solution. Ainworth and Gillespie (2008) recommended a F-C procedure for estimating total phenolic content of plant material using 95% methanol extracts as the starting point. The authors claimed that their method which included a 48 h extraction period reduced interfering substances, such as ascorbic acid (which reacts strongly with the F-C reagent) by 85%. In this thesis research, a 30 min extraction was performed. Given the very high levels of phenolic compounds in wheat bran (Table 2.2) and the extraction procedure used, the estimate of TPC is considered to be reliable.

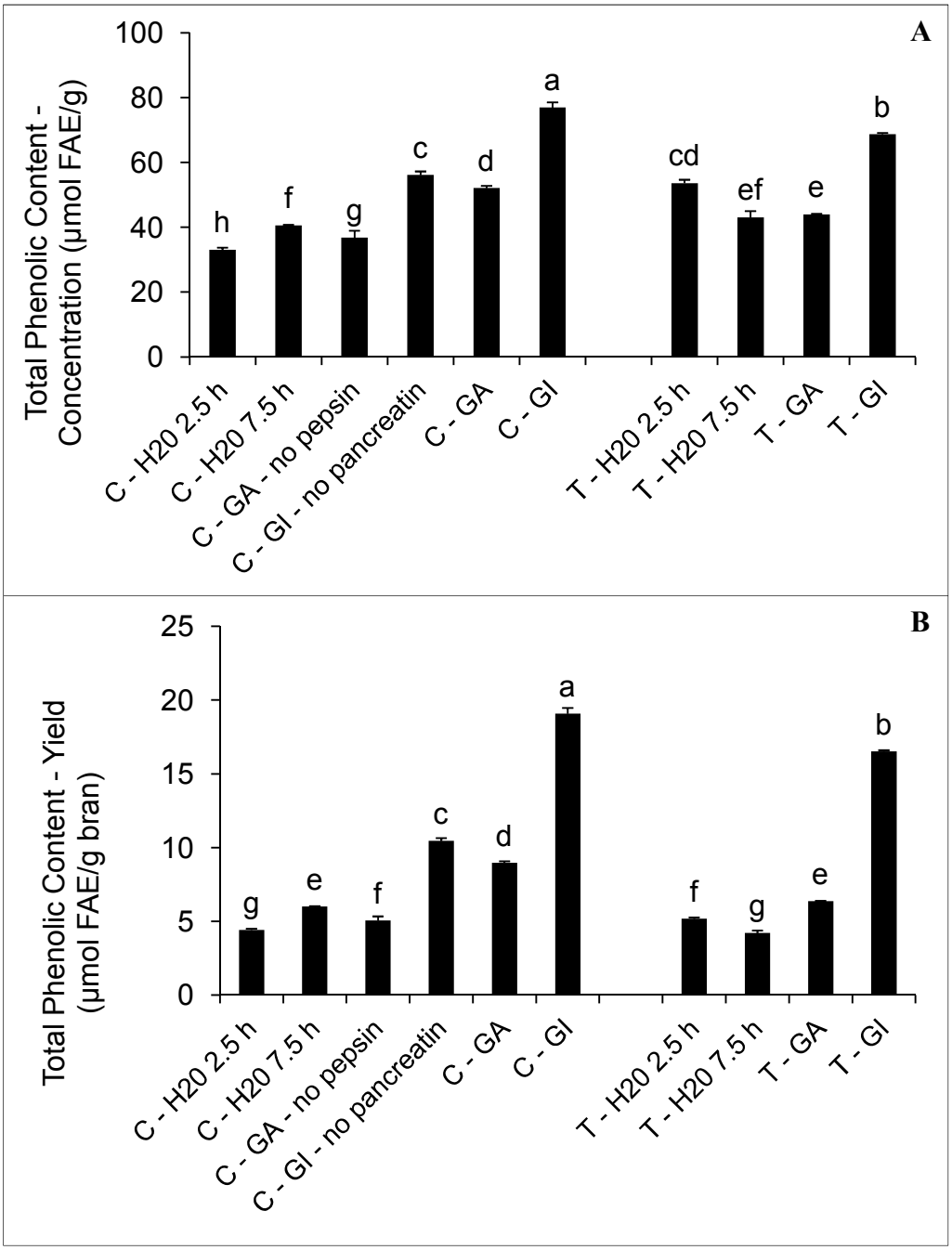


Figure 4.19. CWRS total phenolic content concentration (A) and yield (B) in *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, also 2.5 h and 7.5 h water extraction of control (C) and autoclaved bran (T). Bars with different letters are significantly different ($P < 0.05$).

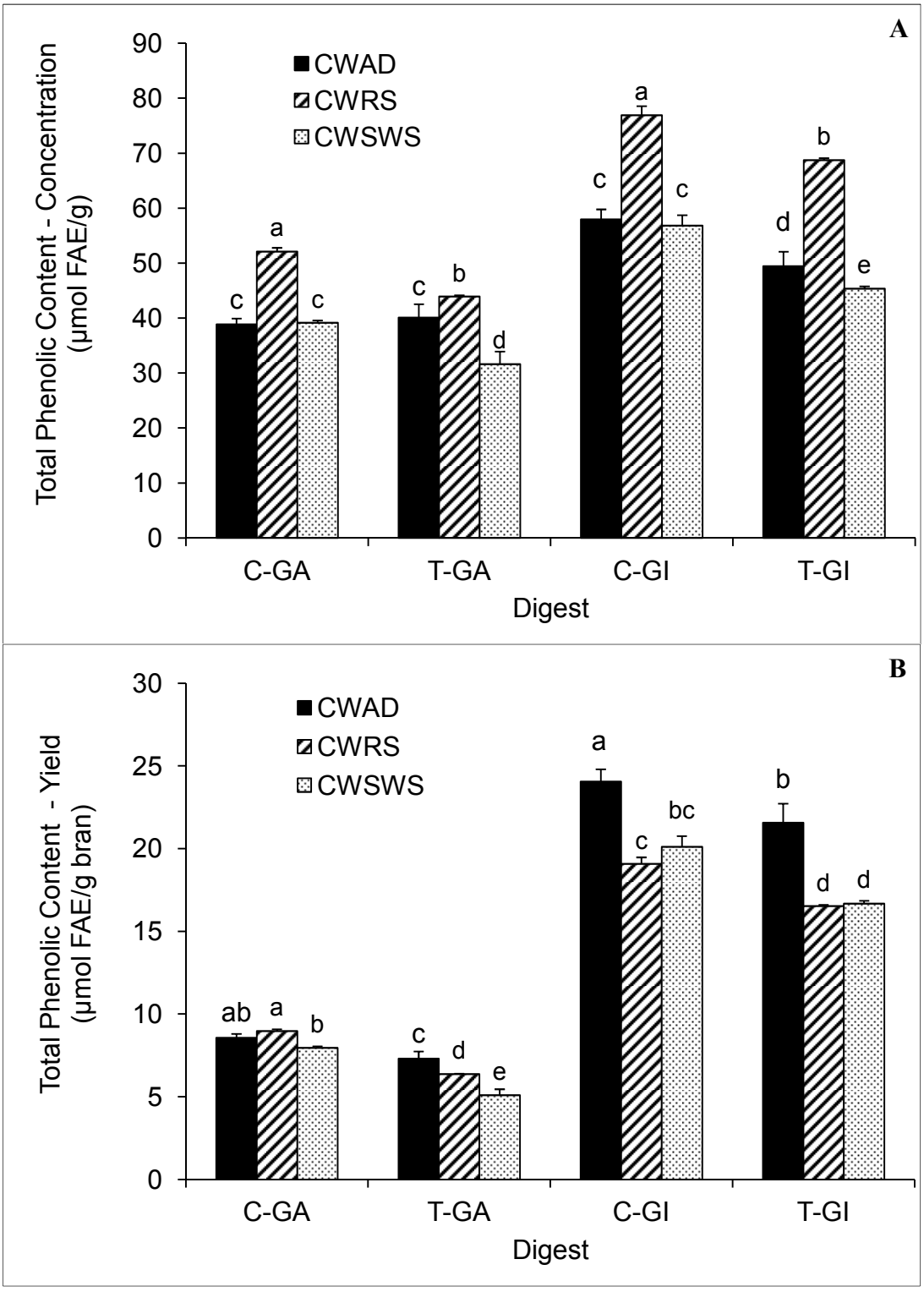


Figure 4.20. Total phenolic content concentration (A) and yield (B) in *in vitro* gastric (GA) and gastrointestinal (GI) digests of control (C) and autoclaved bran (T). Bars with different letters within each digest grouping are significantly different ($P < 0.05$).

4.3.10. Comparison of total phenolic content and antioxidant activity

Linear correlation was assessed for concentration and yield results of TPC vs. DPPH radical scavenging activity, TPC vs. metal chelating activity, and DPPH scavenging activity vs. metal chelating activity. These comparisons were carried out for digests and water extracts of CWRS samples only, as the starting point of analysis was the same bran (Fig.4.21 & 4.22). Comparisons were not done among the bran types to avoid potential confounding issues due to milling of the different wheats resulting in brans with different endosperm, aleurone and pericarp contents.

The correlations for concentration results for the three variables ranged widely from $R^2$ values of 0.17, 0.37 and 0.83 for TPC vs. metal chelating activity, TPC vs. DPPH radical scavenging activity, and DPPH scavenging activity vs. metal chelating activity, respectively (Fig. 4.21). Thus for concentration results, there was no relationship between TPC and antioxidant activity measures. However, the relationship between the two measures of antioxidant activity (free radical scavenging and metal chelation) was strong.

For yield results (Fig. 4.22), correlations among all factors were much higher and ranged in $R^2$ from 0.73 to 0.95 (Fig. 4.22). It should be noted that the four lowest values plotted in these charts correspond to water extracts of the brans as opposed to GA or GI digests. Eliminating these data from the correlation analysis involving TPC, resulted in correlations (for yield) between TPC and DPPH radical scavenging activity and TPC and metal chelation activity of $R^2$=0.90 and 0.99, respectively. One can

legitimately conclude that for digest yields, TPC as determined by the Folin-Ciocalteu method, was an excellent predictor of antioxidant activity.

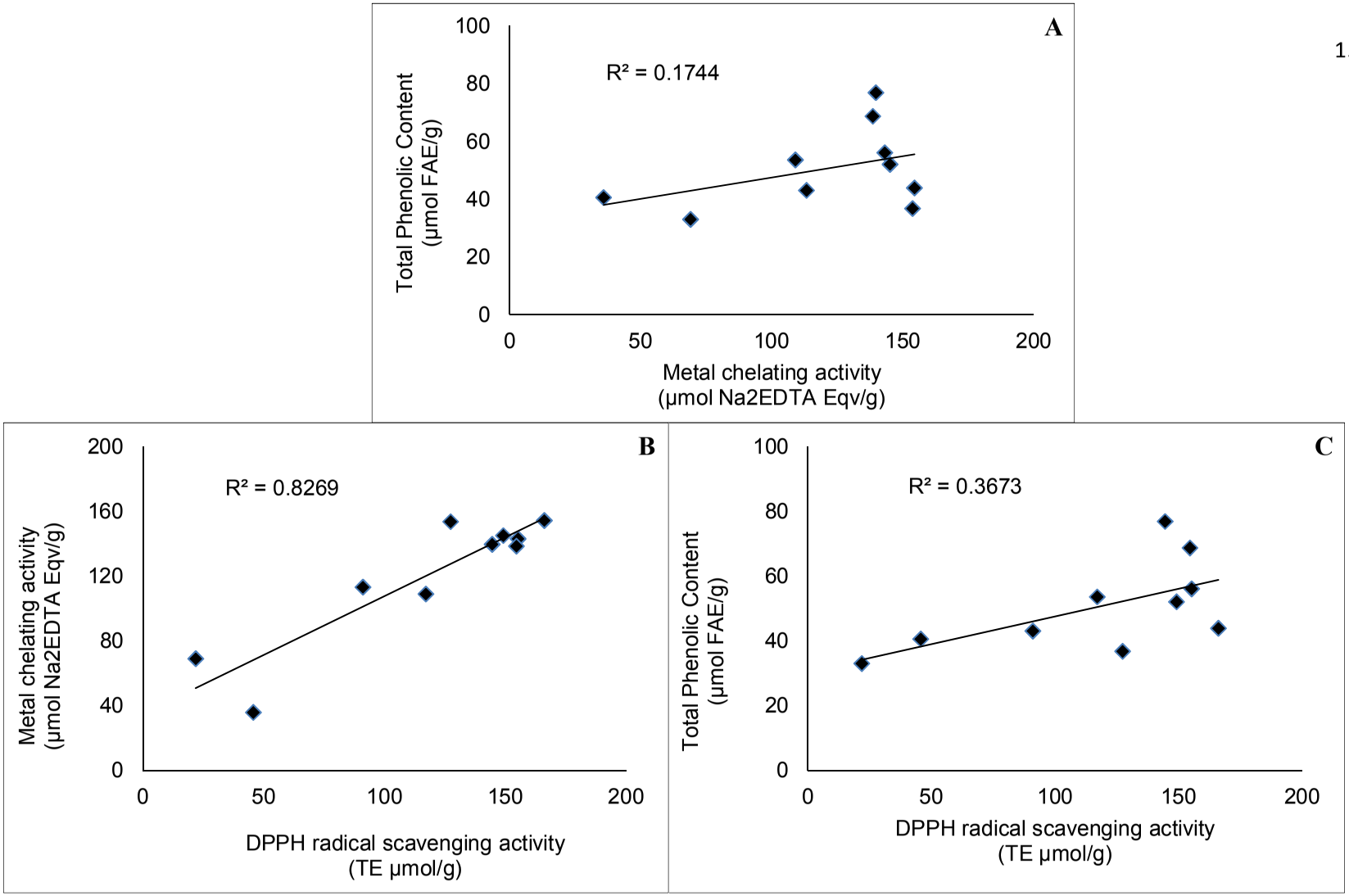


Figure 4.21. Linear correlation of concentration results of CWRS *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, and 2.5 h and 7.5 h water extracts of control (C) and autoclaved bran (T). Total phenolic content versus Metal chelating activity (A), Metal chelating activity versus DPPH radical scavenging activity (B), and Total phenolic content versus DPPH radical scavenging activity (C).

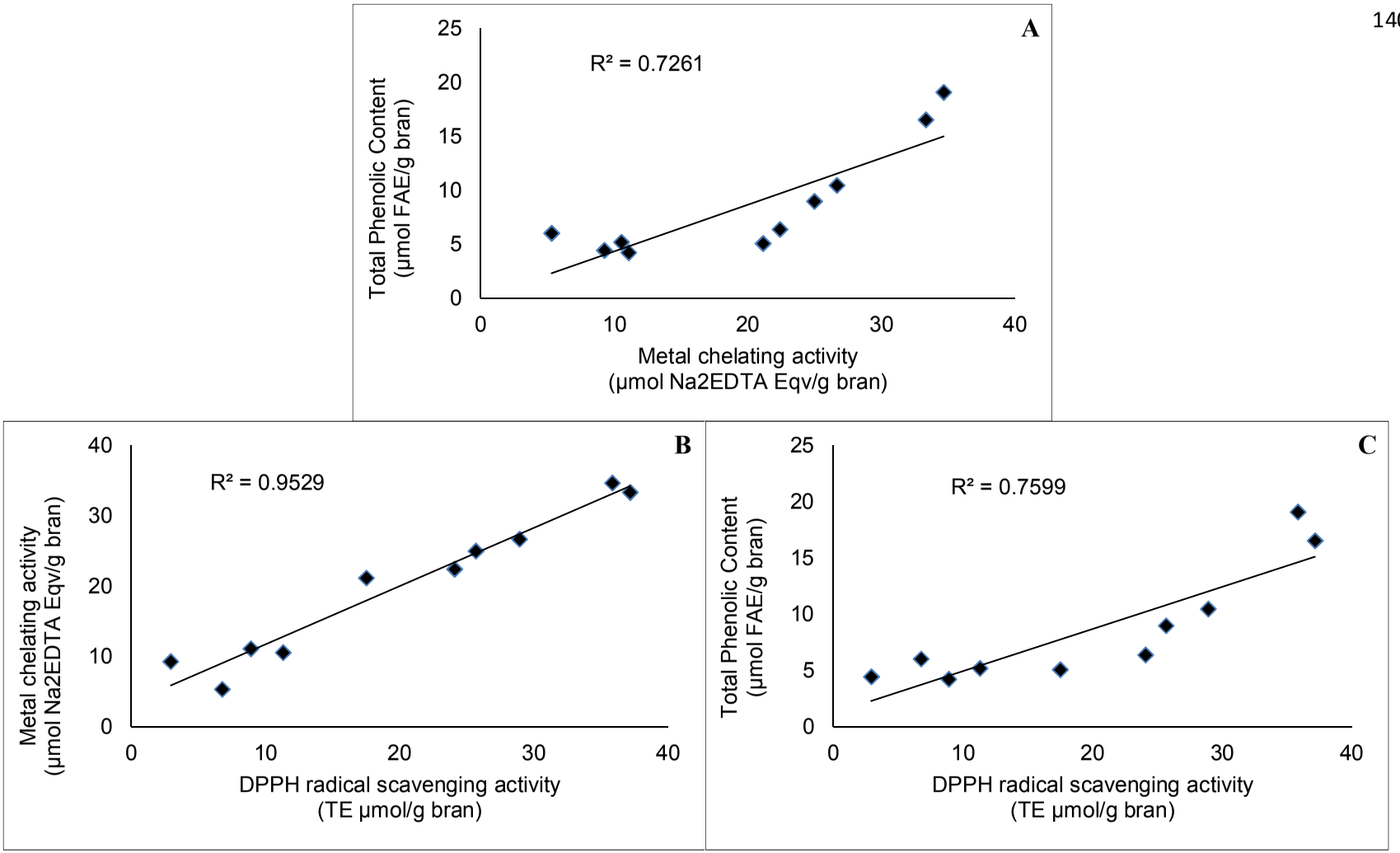


Figure 4.22. Linear correlation of yield results of CWRS *in vitro* gastric (GA), gastrointestinal (GI), GA with no pepsin, and GI with no pancreatin digests, and 2.5 h and 7.5 h water extracts of control (C) and autoclaved bran (T). Total phenolic content versus Metal chelating activity (A), Metal chelating activity versus DPPH radical scavenging activity (B), and Total phenolic content versus DPPH radical scavenging activity (C).

# 5. DISCUSSION

This thesis research contained four objectives that all revolved around the bioaccessibility/extractability of wheat bran as a model of digestibility. The first objective was to develop an *in vitro* digestion model for wheat bran that could reasonably predict the digestion products that are produced during human physiological conditions. Coupled with the first objective was to determine the *in vitro* digestion effect on selected wheat bran nutrient and phytochemical components. The second objective was to determine the effects of *in vitro* digestion phases (GA and GI) on the release of wheat bran nutrients and phytochemical components, i.e. to determine their digestibilties. The third objective of this research was to determine the influence of thermally treating wheat bran in an autoclave on the bioaccessibility outcomes. The fourth objective of this research was to determine if wheat variety or class contributed to differences in bran digestibility.

The *in vitro* digestion model developed for this research was a multiple step static procedure that simulated the upper gastrointestinal tract of humans. The stomach and small intestine were the two sections isolated for construction of this model. The mouth step was omitted from the model because of the lack of significance that salivary α-amylase and mastication would have on wheat bran of fine particle size (Barret, 2006c). The small intestine was used as the cut-off for digestion because most absorption of nutrients takes place in the small intestine (Brandon et al. 2006).

Overall bran digestibility varied from about 29 to 48% (based on GI digests) for non-autoclaved brans depending on the bran type, i.e. wheat class. This is in agreement for *in vitro* digestibility of brans of 29% (Karppinen et al. 2000) and 40% (Woods et al. 2002) using models simulating the upper GI tract. In contrast, lower overall bran digestibility of 13% was reported by Amrein et al. (2003). It is not clear why the level reported by Amrein et al. (2003) was low; unfortunately the authors did not specify the concentration or activity of enzymes used in that study, which could have been lower than optimal. All three studies cited used different methods of *in vitro* digestion in terms of digestions times, enzyme levels, and other variables.

Protein digestibility for *in vitro* GI digestion of the three control wheat brans ranged from 52.6 to 72.2% (Table 4.2). Simulated digestion performed by Amrein et al. (2003) and Wood et al. (2002), using wheat bran fractions found protein digestibility levels of ~ 66 to 70% and 50% for aleurone-rich wheat bran and regular wheat bran, respectively. Amrein et al. (2003) and Wood et al. (2002) both used an *in vitro* model that had a gastric phase of only 30 min, which based on Table 2.3 appears to be less than adequate for the 15 to 30 g of wheat bran they used and therefore may result in less protein digestibility. Karppinen et al. (2000) used a different *in vitro* digestion model which resulted in protein digestibility of 37% for wheat bran. Possible reasons for the low protein digestibility in that study include a relatively reduced time (i.e. 3 h) for the small intestine phase and a third of the amount of pancreatin used compared to the digestion model utilized for this thesis research (Aura et al. 1999). Elsewhere, *in vivo* upper GI tract digestibility of crude protein for pigs consuming a wheat bran diet and a high fibre diet was reported to be 73.3 and 70.9%, respectively (Meunier et al. 2008).

Starch digestibility for *in vitro* GI digestion of the three control wheat brans ranged from 39 to 42% (Table 4.2). These levels are considerably lower than those reported elsewhere (see below) and may have occurred because the digest soluble extract was analyzed rather than the digest residue. Previous studies of starch digestibility analyzed the initial starch content in bran and the starch remaining in bran after digestion. This could explain the difference in digestibility obtained in this thesis research as some starch could be lost or not accounted for in the liquid phase of bran residue or the pellet from the centrifugation step, thus underestimating starch digestibility. . Amrein et al. (2003) and Wood et al. (2002) reported much higher levels of starch digestibility of wheat brans of 90 to 95% when using an initial mouth salivary enzyme step (15 min). Karppinen et al. (2000) found a similar wheat bran starch digestibility of 90%, which agreed with observations of Cummings and Englyst (1991) that in Western diets, close to 10% of all starch is resistant to digestion. *In vivo* upper GI tract starch digestibility levels for pigs were found to be 87.7 and 89.8% for wheat bran and high fibre diets, respectively (Meunier et al. 2008).

While levels of starch digestibility in wheat bran were lower than what has been reported elsewhere, the substantial increase of extracted "starch" (most likely dextrins) after the pancreatin enzyme mixture was added to the GI digests (Fig 4.8B) does demonstrate the ability of the *in vitro* model used in this thesis research to mimic physiological conditions even without salivary α-amylase. Pancreatic α-amylase has a much more important role in starch digestion compared to salivary α-amylase, which only initiates starch digestion (Tavakkolizadeh et al. 2010).

Ash digestibility (GI phase) of the control wheat brans in this research ranged from 42.0 – 53.1% (Table 4.2). This result indicates that upper GI tract digestion is not able to fully release minerals contained in wheat bran. Those minerals are most likely bound in phytin granules of the aleurone layer (Grundas and Wrigley, 2004). A somewhat lower ash digestibility of 33.4% was reported by Amrein et al. (2003), who used a three step *in vitro* digestion model which also indicated that a substantial proportion of minerals in wheat bran are not available for absorption before leaving the small intestine. The higher digestibility values for ash in the present research compared to that of Amrein et al. (2003) could arise from the longer 7.5 h digestion time, as Amrein et al. (2003) used only 3.75 h digestion. A digestion time of 3.75 h is likely suboptimal for human digestion of diets containing wheat bran (Table 2.3). Accordingly, the ash digestibility reported by Amrein et al. (2003) may be an underestimation.

The general conformity of protein, starch and ash digestibility results to physiological conditions illustrates the ability of the *in vitro* model used in this thesis research to mimic wheat bran digestion, and therefore satisfies this main objective of the thesis research.

The extractability of fibre from wheat bran by *in vitro* digestion was measured in this study on the basis of determinations of SDF (SDF = TDF for digests, as IDF content was negligible), pentosan, and β-glucan content of digests. Extractability of SDF (CWRS wheat bran only), pentosan, and β-glucan in GI digests for the three control wheat brans in this study were found to be 1.5% (CWRS only), 2.3 to 3.8%, and 12.4 to 15.6%, respectively (Table 4.4). Similar fibre extractability values for pentosans and β-glucans of 0% and 3% were reported by Karppinen et al. (2000) who did not measure

overall fibre solubility. Wood et al. (2002) subjected a wheat bran fraction ("WBB") prepared by a friction and abrasion milling process to *in vitro* digestion (as well as *in vitro* fermentation). This WBB material comprised ~18% starch, 14% protein, 45% TDF, 19% pentosan and 2.7% β-glucan, which was somewhat similar to the bran used in the present study, except that WBB also contained 12.5% SDF so was much higher in SDF compared to the 1.8% SDF of the CWRS bran in the present study. Accordingly WBB likely reflected a more soluble form of fibre. Results were consistent with this view as calculated extractabilities based on data supplied by Wood et al. (2002) were 8.1%, 13.3%, and 22.2% for TDF, pentosan, and β-glucan, respectively which are much higher than corresponding results obtained in this thesis research. Calculation of fibre and fibre component extractabilities based on data presented by Amrein et al. (2003) showed (implausibly) that there was no change in wheat bran TDF, pentosan, and β-glucan extractabilities due to *in vitro* digestion of wheat bran. This result may be due to the low overall bran extractability reported of 13%. The same digestion model used by Karppinen et al. (2000) applied to wholemeal wheat bread found fibre extractabilities for TDF, pentosan, and β-glucan of 13%, 16%, and 3%, respectively (Aura et al. 1999). These results taken together indicate that wheat bran digested under simulated physiological conditions highly resists fibre release. In contrast, fibre isolated from wheat endosperm, e.g. pentosans is more likely to be much more extractable in the GI tract as they are about 25% water-extractable. In contrast, wheat bran pentosans are ~ 4.5% water soluble (Saulnier et al. 2007; Hashimoto et al. 1987).

Anson et al. (2010) studied the bioaccessibility of antioxidants after 6 h of *in vitro* digestion using the TIM *in vitro* digestion model. Those results, though different

from this study due to the use of a different free radical (i.e. ABTS cf. DPPH radical), showed a steady increase of antioxidant release over the digestion time (Rufian-Henares and Delgado-Andrade, 2009; Anson et al. 2010). Rufian-Henares and Delgado-Andrade (2009), using an *in vitro* digestion model modified after Miller et al. (1981), obtained for fibre-enriched corn breakfast cereals, DPPH scavenging activities of 2.6, 4.0, and 0.5 µmol TE/g of raw cereal, soluble fraction, and insoluble fraction, respectively. The present study resulted in considerably higher antioxidant activities of 30.1 to 37.3 µmol TE/g for control bran GI digests and 18.2 to 23.2 µmol TE/g for GA digests; results may be attributed to greater efficiency of DPPH extraction using 50% acetone (vs. methanol) and structural differences in the corn matrix compared to wheat bran (Zhou and Yu, 2004). Purple wheat bran extracted with ethanol:HCl (85:15, v/v) had DPPH radical scavenging activities of 1.47 mg TE/g (Li et al. 2007), which is 3.8 and 5.9 times lower than that for GA and GI digest of control bran, respectively. The total DPPH radical scavenging activity of wheat bran determined by Miller et al. (2000) was 85.0 µmol TE/g. The average (across bran types) DPPH radical scavenging activity in GA-C and GI-C digests in the present research was 26.3% and 40.4% of those levels, respectively.

Another measure of antioxidant activity is metal chelation. Liyana-Pathirana and Shahidi (2007) reported values for CWRS and CWAD wheat bran, extracted using 80% ethanol for 16 h at 4 °C, of 1316 and 1276 µg EDTA/g, respectively which was considerably lower than values obtained in this thesis research for GA and GI control digests; metal chelating activities ranged from 9,291 to 9,507 and 12,890 to 13,542 µg EDTA/g, respectively for GA and GI control digests of CWRS and CWAD wheat bran. These metal chelating activity results are clearly considerably higher than those reported

for wheat grain and bran based on solvent extraction with e.g. water, 50% acetone, or 100% ethanol (Moore et al. 2006; Zhou et al. 2004a; Zhou et al. 2004b; Liyana-Pathirana and Shahidi, 2005; Yu et al. 2002). By comparison, based on antioxidant tests used in this thesis research, release of wheat bran antioxidants during simulated digestion is markedly boosted when bran is exposed to physiological digestion conditions that include pH changes, enzymes and longer resident times for bran in the digest media.

The antioxidant activity of cereal brans should reflect the content and composition of phenolic constituents, particularly phenolic acids which make up the majority of bran phenolics (Table 2.2). However, correlations between total phenolic content (typically determined by the Folin method) and antioxidant activities are often very weak (Verma et al. 2008). The average TPC (hydrolyzed by 2 M NaOH) and free phenolic content for wheat brans of 51 cultivars was reported to be 5932.2 and 1493.0 FAE μg/g (80% ethanol extracted for 10 min at RT), respectively (Verma et al. 2008). By comparison, the average TPC of GA-C and GI-C digests in the present study was 27% and 69% of those values, respectively. This result is plausible as it would be unlikely that *in vitro* digests of wheat bran would release the same levels of TPC extracted by NaOH hydrolysis. Liyana-Pathirana and Shahidi (2007) reported TPC for CWAD and CWRS wheat bran (extracted with 80% ethanol at 4º C for 16 h) of 2279 and 3437 μg FAE/g, respectively. Compared to the CWAD and the CWRS control brans analyzed for TPC in this thesis research, those results rank in between the GA and GI digests for both brans.

As mentioned previously in Results, in principle, the Folin-Ciocalteu procedure is not absolutely specific for phenolic compounds (Liyana-Pathirana and Shahidi. 2005; Singleton et al. 2009). A number of compounds could interfere with accurate phenolic compound quantification such as ascorbic acid and sugars. Also as previously mentioned, overestimation may arise from non-phenolics such as proteins (Liyana-Pathirana and Shahidi. 2005) and more specifically aromatic amino acids and cysteine (Singleton et al. 2009). While these amino acids can be expected to be found in proteins of wheat bran, extracting *in vitro* digests with 100% methanol and for 30 min for the F-C method should have been sufficient to eliminate or at least highly minimize any proteins from solution as well as other confounding compounds. Given the very high levels of phenolic compounds in wheat bran (Table 2.2) and the extraction procedure used, the estimate of TPC is considered to be reliable. This assertion is supported by the TPC yield results obtained in the present research which, for CWRS bran digests and water extracts, appeared to follow very similar patterns of variation seen for antioxidant activity yield as measured by DPPH radical scavenging and metal chelating activity (compare Figs. 4.15B, 4.17B and 4.19B). This assertion is also supported by the high correlations obtained between TPC and DPPH scavenging activity and TPC and metal chelation activity for yield results (Fig. 4.22).

In comparison to other *in vitro* and human bioavailability studies, the high TPC content (FA equivalents) found in this *in vitro* digestion procedure supports the abundance of wheat bran antioxidants found in the digests. Anson et al. (2009b) using a whole-meal bread with native wheat bran sample on the TIM system found a maximal upper intestinal FA absorption of ~ 0.8 μmol/g (compared to 20.1 to 24.1 μmol FAE/g

bran for GI-C digests). Kern et al. (2003) reported maximal human plasma FA concentrations to be ~ 200 nM when consuming 100 g of a high-bran breakfast cereal (wheat) (compared to 2.0 to 2.4 mM FAE/100 g bran for GI-C digests).

Wheat bran contains fibre (e.g. cellulose, pentosans, and lignin) that have demonstrated the ability to bind minerals (Camire and Clydesdale, 1981; Claye et al. 1996: Anglani, 1998). So, the consistently lower NaCl yield of GA digests compared to GI counterparts (Fig 4.3) could likely be the result of greater breakdown of fibre occurring over the longer incubation time of the GI phase which would results in greater release of minerals bound to insoluble fibre.

The effect of physiological conditions both GA and GI phases vs. simple water extractions (2.5 and 7.5 h) for the control CWRS bran was clearly apparent for the extraction yields of many wheat bran components. Yields of ash, protein, β-glucan, DPPH scavenging activity, metal chelating activity, and TPC were noticeably and significantly higher for control GA digests compared to 2.5 h water extracts. The higher yields of ash, protein, and β-glucan may be attributed to both the pepsin and acidic conditions. The DPPH scavenging activity, metal (iron) chelating activity, and TPC increase in the GA-C-no pepsin digest compared to simple water extraction, points to acidic conditions as the major factor. Arranz and Calixto (2010) noted that weak acidic conditions like those used in simulated gastric digestion may completely release soluble polyphenols, while only partially releasing insoluble bound polyphenols. Liyana-Pathirana and Shahidi (2005) found similar pH-related enhancement of TPC, and antioxidant activities (DPPH radical scavenging and metal chelation) for bran of hard and soft wheats. Baublis et al. (2000) also observed increases in antioxidant activity

when cereals containing high-bran content and whole grains were exposed to an acidic pH. Similarly, acidic pH treated CWRS wheat bran compared to untreated bran produced 2.4X, 2.6X, and 3.3X increases in TPC, DPPH scavenging activity and metal (iron) chelating activity, respectively (Liyana-Pathirana and Shahidi, 2005). In the present study, GA-no pepsin digests of CWRS bran increased TPC, DPPH scavenging activity and metal (iron) chelating activities by 1.1X, 6.0X, and 2.1X those of simple water (2.5 h) extracts. The large difference in DPPH radical scavenging activity between the current study and the two cited studies for pH effects, may be due to the use of 50% acetone solvent (a highly effective solvent) and that a freeze dried digest (directly dissolved in DPPH solution) was used for extracting wheat bran antioxidants (Zhou and Yu, 2004). There were also expected increases for the GI-C digest compared to the water extraction for 7.5 h. Yields of protein, starch, pentosan, β-glucan, TPC, DPPH scavenging and metal chelating activity yield were considerably increased using simulated physiological conditions of the GI digestion phase.

There was a consistent and significant increase in yield of all nutrient and phytochemical components (except SDF) for the GI digest compared to GA counterpart. The increase in yield can be at least partly attributed to the longer extraction time of the GI vs. GA phase of 2.5 and 7.5 h, respectively. One of the main factors affecting simulated digestion is the incubation time (Hur et al. 2011). A 5 h increase in extraction time likely provides more time for the further release of bran components, as was seen for pentosans, DPPH radical scavenging activity, and metal chelating activity when comparing the control CWRS GA digest with the CWRS GI-no pancreatin digest. The presence of pancreatin enzyme in the GI digest phase is another important factor

creating a difference between GA and GI results. The pancreas secretes the majority of the body's digestive enzymes for carbohydrates, proteins, and lipids required for adequate digestion and absorption (Barret, 2006d). Pancreatic enzymes are produced in excess of that required for digestion, so the increase in digestibility of enzyme-labile bran constituents would be expected when using activity levels that match *in vivo* conditions for the *in vitro* model (Table 2.5) (Barret, 2006d). Amrein et al. (2003) determined through microscopic analysis of *in vitro* digested aleurone cells, that cell walls are likely ruptured due to osmotic pressure (via cell mineral content) and possibly endogenous enzymes. It can be assumed that ruptured cells in bran tissue would release more cell components when exposed to a longer GI extraction time (7.5 vs. 2.5 h) and pancreatic enzymes.

The effect of thermally treating wheat bran by autoclaving was another aspect of this thesis research. In recent research by Dona (2011), autoclaved CWRS wheat bran was extracted with water for increasing times up to 18 h, and the solubility of numerous bran constituents were monitored. In the present research, antioxidant activity (DPPH scavenging activity) of water extracts of autoclaved bran was similarly increased. Also, analogous to Dona (2011) concentrations of protein, starch, and ash levels were reduced in water extracts of autoclaved bran compared to control bran. Autoclaved wheat bran in this thesis research produced lower TPC levels compared to Dona (2011), which may be the result of lower extraction times (2.5 and 7.5 h vs. 18 h), higher extraction temperature (37º C vs. RT), and/or different extraction solvents (100% methanol vs. acidified methanol) (Dona, 2011). The GA digests for all bran components that experienced significant changes in yield as a result of autoclaving treatment were

protein (↓ 40.4%), TPC (↓26.6%), and SDF/TDF (↑ 88.2%). The GI digests for all bran components that produced significant differences in yield as a result of autoclaving were protein (↓ 13.3%), pentosan (↑ 28.8%), β-glucan (↑ 21.8%), TPC (↓ 13.6%), and SDF (↑ 76.8%). Significant increases in autoclaved digest yields that were not uniform among all wheat brans in a digest type were found for ash (GI, CWAD and CWRS) starch (GA, CWAD and CWSWS), pentosan (GA, CWRS), β-glucan (GA, CWSWS and CWAD) and DPPH scavenging activity (GA and GI, CWAD), metal chelating activity (GI, CWAD). The level of SDF increase for autoclaved wheat bran digests remains to be fully determined. Dona (2011) found concentrations of resistant oligosaccharide (an SDF component) in water extracts of autoclaved bran extracts to be considerably increased compared to untreated bran counterparts. This unaccounted for SDF fraction is a likely candidate to make up a large part of the missing fraction of GA and GI digests yet to be clarified (Table A.6).

The decrease in protein in digests/extracts of autoclaved wheat bran may be the result of Maillard reactions and/or denatured proteins that become insoluble in subsequent extractions ($H_2O$ and *in vitro* digestion). The increases in pentosan, β-glucan, and SDF in GI digests of autoclaved bran, further demonstrates the ability of this thermal treatment to physically breakdown cell wall components (Caprez et al. 1986; Lebet and Amado, 1998; Saulnier et al. 2001). Noted increases in pentosan and β-glucan content of GI digests in contrast to GA digests of autoclaved bran could be the result of the longer exposure to physiological conditions of the former compared to the latter digest. The substantial increase in antioxidant activity of water extracts of autoclaved bran seen by Dona (2011) was not found in the *in vitro* digests of this study.

It may be the case that digestion of normal wheat bran can effectively liberate antioxidants (phenolic compounds and phytic acid) without the need for any thermal pre-treatment. HPLC of individual phenolic acids, ferulic acid most notably, of digests of autoclaved and control bran would be needed in order to clearly determine the effect, if any, of this thermal treatment.

The effect of wheat cultivar (i.e. class) on wheat bran phytochemical and nutrient yield was apparent for yields of protein, starch, pentosan, β-glucan, and TPC. Digestion of CWAD wheat bran, compared to CWRS and CWSWS brans, released significantly more protein (GA and GI), starch (GI), TPC (GA-T, GI-C, and GI-T), and DPPH radical scavenging activity (GA-T and GI-T). The high starch and protein levels found for the CWAD wheat digests can be attributed to the roller milling process as mentioned earlier. Very high starch (i.e. endosperm) content was found in CWAD wheat bran despite the very long tempering times (72 h) used. Evidently the CWAD wheat bran did not absorb enough water for adequate separation of bran from endosperm. The high protein yield and extractability of CWAD wheat bran digests is likely attributable to the same effects of adhering endosperm, as it is well known that an increasing gradient of protein exists in wheat kernels from inner to outer endosperm. CWAD wheat bran does not characteristically possess higher levels of total phenolics than other wheat brans like hard common and soft wheats (Liyana-Pathirana and Shahidi, 2007; Verma et al. 2008; Adom et al. 2003). High TPC yield found in the CWAD wheat GI-C and GA-T digests did not translate into higher antioxidant activities for the metal chelating antioxidant test, as it did for DPPH radical antioxidant activities of the GA-T and GI-T digests. The lack of association between total phenolic content and antioxidant activity of the

CWAD wheat GI digest's could again be attributed to the Folin reagent's high susceptibility towards interference from reducing agents present in wheat bran and/or an abundance of phenolics with relatively weak antioxidant strength (Verma et al. 2008). Without further HPLC analysis, the effect of wheat bran genotype or class on extractability of phenolic compounds due to digestion is uncertain.

Digest components of CWSWS wheat bran, i.e. pentosan (GA-C, GI-C, and GI-T) and β-glucan (GA-T, GI-C, and GI-T) were extracted in amounts significantly higher than in the other wheat bran classes analyzed. The noticeably higher pentosan and β-glucan contents of GI digests of CWSWS bran may be associated with different bran structural characteristics that would make it more susceptible to physiological conditions. This of course assumes that the bran sample(s) used in this thesis research was representative of wheat brans of similar types. In this regard, results were more than likely representative of true class differences as each of the three brans were derived from large-scale commercial composites of wheat derived from many different producers over a broad geographical region in Western Canada.

Another apparent difference due to bran class/type was observed for ash/mineral levels of CWRS wheat digests that were consistently higher than those for the CWAD and CWSWS bran digests. The likely reason for this is the lower endosperm/starch content that is typical for CWRS wheat bran which would result in a higher proportion of mineral rich bran (Table 4.1). This and other results reported in this thesis underscore the importance of determining starch content of bran when bran is the subject of functional food studies. It is noteworthy that this piece of information, i.e. starch content

reflecting the purity of bran fibre and phytochemical constituents has only infrequently been reported in the literature in similar studies.

This discussion of differences in digest analyte results among different bran types points to an issue of confounding effects arising from milling of wheats that are inherently different in milling performance, most likely due to differences in hardness or density, resulting in brans with different composition of "contaminating" starch (i.e. endosperm) and therefore different concentrations or purity of aleurone and pericarp tissue. It is therefore recommended in future research on digestibility differences among wheat classes, that material for analysis should comprise ground samples of whole wheat instead of bran to accurately determine wheat/bran type effects.

# 6. CONCLUSIONS

The *in vitro* digestion model used in this research demonstrated its capability to closely match *in vivo* digestibility values of starch and protein for pigs, which possess similar physiological conditions as humans. The *in vitro* digestion model possessed high extractability values for protein, starch, and ash components in wheat bran, while relatively low extractability results were found for pentosans, β-glucan and SDF, i.e. fibre in general. High extractability values for certain components can most likely be attributed to the digestive enzymes and pH conditions used, while the low extractability values can be attributed to wheat bran's rigid structural properties which is stabilized by cell walls containing high levels of non-starch polysaccharides. Antioxidant activity and TPC content was significantly enhanced by *in vitro* digestion compared to water extracts, which indicates that physiological digestion conditions are able to produce appreciable levels of bioaccessible antioxidant components. For future studies, the use of the GA digestion phase can be omitted, as it was incorporated into this thesis research to determine the effectiveness of the *in vitro* digestion procedure. The GI digestion proved to be satisfactory in mimicking the upper gastrointestinal conditions.

The GI phase for almost all wheat bran nutrient and phytochemical components (except SDF) extracted more than the GA phase during *in vitro* digestion. The likely reasons are the hydrolyzing effects of pancreatin and a longer extraction time (7.5 h vs. 2.5 h).

The wheat bran autoclave pre-treatment used in this research had varied effects for GA and GI digests. While the GA digest was only significantly enhanced in SDF

content due to autoclaving of bran, the GI digest showed increased levels of pentosan, β-glucan, and SDF. The increase in fibre extraction levels can be explained by the physical breakdown of wheat bran cell walls caused by autoclaving. The increase in fibre-related levels for pentosan and β-glucan in the GI digest cf. GA digest could be credited to the longer incubation time for the GI phase, to further extract constituents from damaged cell walls arising from autoclaving. The effects of wheat bran class on extractability results due to *in vitro* digestion appear to be practically significant as results for CWAD and CWSWS wheat brans were very noticeably different from those of CWRS bran. CWAD wheat bran's high yield of starch (GA) and protein (GA and GI) could be attributed to the very hard kernel characteristic of this wheat class causing significant adherence of endosperm (i.e. starch) of high protein content, notwithstanding long tempering times, that resisted the normally satisfactory separation of bran from endosperm typical for hard common wheats in roller milling. CWAD wheat bran also had higher TPC (GA-T, GI-C, and GI-T) and DPPH radical scavenging activity levels (GA-T and GI-T). High CWSWS wheat bran fibre yields for pentosan (GA and GI) and β-glucan (GI) could be attributed to unidentified structural aspects in the bran.

Recommendations for future research include more specific analysis of phenolic content and composition of wheat brans subjected to *in vitro* digestion. The need to elucidate the effects of wheat class and possibly even genotype on the physiological release of wheat bran nutrients and phytochemicals during digestion would be an important addition to knowledge in order to maximize the benefits of wheat bran in food products for health-conscious consumers.

In conclusion, results taken as a whole indicate that the *in vitro* digestion protocol used in this thesis research was a suitable and effective procedure to study the effects of simulated digestion on wheat bran. As would be expected, release of nutrients from insoluble bran, due to *in vitro* digestion, such as minerals, protein and starch was substantial in contrast to the lesser, but still significant, effects on fibre (SDF) and fibre fractions such as pentosans, β-glucans. Perhaps the most notable outcome of the research was the effect of digestion on factors related to antioxidant activity of digests. In contrast to the relatively small increases in the solubility of fibre due to *in vitro* digestion, total phenolic content, free radical scavenging activity and metal chelation activity were all substantially increased by more than ~ 5X, ~15X and 5X, respectively, in GI digests of wheat bran compared to simple water extracts. Furthermore, yield levels of these factors suggest that wheat bran, when digested, releases ample levels of antioxidants that should be bioavailable in the small intestine to promote beneficial health effects attributable to bran and its phenolic constituents.

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# 8. APPENDIX

Table A.1. Sample weights, titration volumes, and pHs for recorded for *in vitro* digestions[a]

| Bran | Extraction type | Weight (g) | 6 M HCl (ml) | Starting pH | 3 M NaOH (ml) | End pH |
|---|---|---|---|---|---|---|
| CWAD | GA-C | 100.00 | 31.3 | 2.06 | 25.8 | 6.90 |
| CWAD | GA-T | 100.00 | 31.3 | 2.04 | 26.5 | 6.88 |
| CWAD | GI-C | 100.06 | 33.0 | 2.04 | 26.9 | 6.83 |
| CWAD | GI-T | 100.05 | 31.5 | 2.04 | 26.9 | 6.88 |
| CWRS | GA-C | 100.03 | 39.1 | 2.02 | 31.9 | 6.90 |
| CWRS | GA-T | 99.99 | 38.9 | 2.01 | 32.4 | 6.92 |
| CWRS | GI-C | 100.03 | 40.0 | 2.00 | 33.0 | 6.93 |
| CWRS | GI-T | 99.96 | 40.0 | 2.02 | 33.0 | 6.85 |
| CWSWS | GA-C | 99.99 | 35.0 | 2.02 | 28.0 | 6.88 |
| CWSWS | GA-T | 2.04 | 35.0 | 2.04 | 28.0 | 6.87 |
| CWSWS | GI-C | 99.98 | 35.0 | 2.03 | 28.3 | 6.85 |
| CWSWS | GI-T | 100.01 | 35.0 | 2.02 | 29.0 | 6.88 |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated

Table A.2. Analysis of Variance Mean Squares by General Linear Model Procedure for Wheat Bran *in vitro* Digestion Yield [a,b,c,d]

| Source | DF | Digest Component Yield | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ash | Protein | Starch | Pentosan | β-glucan | DPPH | MCA | TPC |
| Bran | 2 | 1.05E-04*** | 6.47E-03*** | 2.63E-03*** | 1.78E-05*** | 7.73E-06*** | 160.38*** | 26.57** | 20.69*** |
| Treat | 1 | 1.30E-07 | 3.95E-03*** | 4.35E-06 | 8.39E-06*** | 1.02E-06*** | 41.21** | 1.89 | 38.44*** |
| Digest | 1 | 7.42E-05*** | 8.28E-03*** | 2.27E-02*** | 3.12E-05*** | 2.54E-06*** | 1170.23*** | 1197.89*** | 906.66*** |
| Bran*Digest | 2 | 1.17E-06** | 4.15E-04*** | 2.49E-03*** | 1.85E-06*** | 2.70E-07*** | 17.42* | 22.10** | 11.50*** |
| Digest*Treat | 1 | 1.27E-05*** | 6.98E-04*** | 4.50E-07 | 2.58E-06*** | 2.20E-07*** | 22.10* | 57.45*** | 0.49 |
| Bran*Treat | 2 | 4.79E-06*** | 2.14E-05*** | 4.59E-06 | 3.90E-07 | 8.00E-08** | 25.87** | 10.96* | 0.81 |
| Bran*Digest*Treat | 2 | 5.40E-07* | 1.75E-05*** | 2.95E-06 | 2.40E-07 | 2.00E-08 | 2.43 | 11.52* | 0.20 |

[a]DPPH, DPPH scavenging activity; MCA, Metal chelating activity; TPC, total phenolic content;

[b] Ash, Protein, Starch, Pentosan, and β-glucan are expressed in mg/g

[c] DPPH, MCA, and TPC are expressed in umol/g

[d] *, **, and ***, mean square term significant at $P < 0.05$, 0.01, and 0.001, respectively

Table A.3. Analysis of Variance Mean Squares by General Linear Model Procedure for Wheat Bran *in vitro* Digestion Concentration (%)[a,b]

| Source | DF | Digest Component % | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ash | Protein | Starch | Pentosan | β-glucan | DPPH | MCA | TPC |
| Bran | 2 | 77.88*** | 160.46*** | 95.46*** | 4.95*** | 2.28*** | 7343.47*** | 2902.51*** | 662.73*** |
| Treat | 1 | 6.04*** | 452.78*** | 4.55E-02 | 3.24*** | 0.51*** | 2196.31*** | 148.36* | 303.36*** |
| Digest | 1 | 112.34*** | 345.52*** | 1602.35*** | 2.75*** | 1.30*** | 1687.66*** | 2168.79*** | 2000.43*** |
| Bran*Digest | 2 | 0.95*** | 67.65*** | 86.20*** | 0.24** | 3.05E-02*** | 181.13* | 110.85 | 65.66*** |
| Digest*Treat | 1 | 1.93*** | 73.00*** | 0.95 | 0.21** | 9.16E-02*** | 445.00** | 8.65 | 31.67** |
| Bran*Treat | 2 | 1.21*** | 0.28*** | 0.19 | 0.26*** | 1.91E-02** | 173.35* | 38.98 | 18.86** |
| Bran*Digest*Treat | 2 | 1.65E-02 | 1.61*** | 0.15 | 3.68E-03 | 6.06E-03 | 40.38 | 36.59 | 12.04* |

[a] DPPH, DPPH scavenging activity; MCA, Metal chelating activity; TPC, total phenolic content

[b] *, **, and ***, mean square term significant at $P < 0.05$, 0.01, and 0.001, respectively

Table A.4. Shapiro-Wilks test for normality of wheat bran constituent yield[a]

| Digest constituent | 3 wheat bran digests GA | GI | CWRS |
|---|---|---|---|
| Ash | - | + | + |
| Protein | + | + | - |
| Starch | + | + | - |
| Pentosan | + | + | + |
| β-glucan | + | + | + |
| SDF | n/a | n/a | + |
| MCA | + | + | + |
| DPPH | + | + | + |
| TPC | + | + | + |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated; DPPH, DPPH scavenging activity; MCA, Metal chelating activity; TPC, total phenolic content; SDF, soluble dietary fibre;

Table A.5. Shapiro-Wilks test for normality of wheat bran constituent %[a]

| Digest constituent | 3 wheat bran digests GA | GI | CWRS |
|---|---|---|---|
| Ash (%) | + | + | + |
| Protein (%) | + | + | - |
| Starch (%) | + | + | - |
| Pentosan | + | + | - |
| β-glucan | + | + | + |
| SDF | n/a | n/a | + |
| MCA | + | - | + |
| DPPH | + | + | - |
| TPC | + | + | + |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated; DPPH, DPPH scavenging activity; MCA, Metal chelating activity; TPC, total phenolic content; SDF, soluble dietary fibre

Table A.6. Dry matter analyte yield recovery of CWRS wheat bran digests[a]

| Digest Constituents (g/100 g bran) | CWRS wheat bran digests | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GA-C | | GI-C | | GA-T | | GI-T | |
| | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| Ash | 3.05 | 0.04 | 3.24 | 0.03 | 3.07 | 0.02 | 3.52 | 0.06 |
| Protein | 6.66 | 0.04 | 8.27 | 0.00 | 3.65 | 0.01 | 6.89 | 0.04 |
| Starch | 0.10 | 0.01 | 2.40 | 0.05 | 0.12 | 0.00 | 2.23 | 0.19 |
| SDF | 0.76 | 0.06 | 0.78 | 0.03 | 1.44 | 0.08 | 1.37 | 0.03 |
| IDF | 0.00 | - | 0.00 | - | 0.00 | - | 0.00 | - |
| TPC | 0.17 | 0.00 | 0.37 | 0.01 | 0.12 | 0.00 | 0.32 | 0.00 |
| Total analyte yield | 10.74 | | 15.05 | | 8.40 | | 14.34 | |
| Total digest yield | 18.63 | | 28.10 | | 15.57 | | 27.31 | |
| Analyte yield recovery (%) | 57.66 | | 53.57 | | 53.96 | | 52.51 | |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated; SDF, soluble dietary fibre; IDF, insoluble dietary fibre; TPC, total phenolic content (ferulic acid equivalents)

Table A.7. Concentrations of ash, protein, starch, pentosan and β-glucan in *in vitro* digests and $H_2O$ extracts of CWRS wheat brans[a]

| Extraction type | Time (hr) | Digest | Digest Constituents | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ash (%) | | Protein (%) | | Starch (%) | | Pentosan (%) | | β-glucan (%) | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| $H_2O$ | 2.5 | C | 18.35 | 0.21 | 27.37 | 0.10 | 2.77 | 0.09 | 2.86 | 0.31 | 1.67 | 0.03 |
| $H_2O$ | 7.5 | C | 22.02 | 0.02 | 26.99 | 0.05 | 1.65 | 0.09 | 2.07 | 0.20 | 0.86 | 0.05 |
| GA-no pepsin | 2.5 | C | 17.00 | 0.07 | 26.68 | 0 | 0.57 | 0 | 2.65 | 0.07 | 1.49 | 0.01 |
| GI-no pancreatin | 7.5 | C | 13.24 | 0.04 | 38.53 | 0.06 | 0.95 | 0.04 | 3.18 | 0.15 | 1.66 | 0.04 |
| GA | 2.5 | C | 14.85 | 0.17 | 38.67 | 0.23 | 0.58 | 0.04 | 2.62 | 0.12 | 1.68 | 0.04 |
| GI | 7.5 | C | 11.47 | 0.10 | 33.34 | 0 | 9.66 | 0.21 | 2.57 | 0.05 | 1.33 | 0.09 |
| $H_2O$ | 2.5 | T | 11.59 | 0.14 | 12.77 | 0 | 0.85 | 0.07 | 3.91 | 0.26 | 2.77 | 0.03 |
| $H_2O$ | 7.5 | T | 11.83 | 0.06 | 13.48 | 0.05 | 1.07 | 0.09 | 3.22 | 0.10 | 3.06 | 0.10 |
| GA | 2.5 | T | 17.26 | 0.14 | 25.19 | 0.06 | 0.82 | 0.02 | 4.00 | 0.08 | 2.04 | 0.07 |
| GI | 7.5 | T | 12.82 | 0.21 | 28.68 | 0.17 | 9.29 | 0.78 | 3.50 | 0.22 | 1.53 | 0.00 |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated

Table A.8. Concentrations of DPPH radical scavenging activity, metal chelation activity and total phenolic content of *in vitro* digests and $H_2O$ extracts of CWRS wheat bran[a]

| Extraction type | Time (hr) | Digest | Digest Constituents | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | DPPH (μmol TE/g) | | MCA ($Na_2EDTA$ μmol/g) | | TPC (μmol FAE/g) | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| $H_2O$ | 2.5 | C | 21.88 | 0.97 | 69.05 | 2.37 | 33.03 | 0.71 |
| $H_2O$ | 7.5 | C | 45.71 | 2.56 | 35.87 | 1.53 | 40.61 | 0.20 |
| GA-no pepsin | 2.5 | C | 127.33 | 6.34 | 153.73 | 2.45 | 36.80 | 2.15 |
| GI-no pancreatin | 7.5 | C | 155.25 | 1.04 | 143.13 | 5.48 | 56.11 | 1.14 |
| GA | 2.5 | C | 149.15 | 0.60 | 145.11 | 0.45 | 52.10 | 0.70 |
| GI | 7.5 | C | 144.53 | 1.05 | 139.74 | 4.65 | 76.93 | 1.62 |
| $H_2O$ | 2.5 | T | 117.08 | 0.12 | 109.01 | 6.08 | 53.63 | 1.05 |
| $H_2O$ | 7.5 | T | 91.05 | 1.63 | 113.26 | 5.60 | 43.08 | 1.91 |
| GA | 2.5 | T | 166.09 | 14.99 | 154.45 | 1.62 | 43.92 | 0.24 |
| GI | 7.5 | T | 154.57 | 4.82 | 138.58 | 5.24 | 68.74 | 0.35 |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated; DPPH, DPPH scavenging activity; MCA, Metal chelating activity; TPC, total phenolic content

Table A.9. Yields of ash, protein, starch, pentosan and β-glucan in *in vitro* digests and $H_2O$ extracts of CWRS wheat brans[a]

| Extraction type | Time (hr) | Digest | Digest Constituents | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ash (mg/g bran) | | Protein (mg/g bran) | | Starch (mg/g bran) | | Pentosn (mg/g ran) | | β-glucan (mg/g bran) | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| $H_2O$ | 2.5 | C | 24.59 | 0.29 | 36.69 | 0.13 | 3.71 | 0.12 | 3.84 | 0.41 | 2.24 | 0.04 |
| $H_2O$ | 7.5 | C | 32.61 | 0.04 | 39.97 | 0.07 | 2.44 | 0.13 | 3.06 | 0.30 | 1.28 | 0.07 |
| GA-no pepsin | 2.5 | C | 28.92 | 0.11 | 3 68 | 0 | 0.78 | 0 | 3.65 | 0.09 | 2.04 | 0.02 |
| GI-no pancreatin | 7.5 | C | 29.63 | 0.09 | 71.77 | 0.11 | 1.77 | 0.08 | 5.93 | 0.28 | 3.09 | 0.07 |
| GA | 2.5 | C | 30.47 | 0.36 | 66.56 | 0.40 | 1.00 | 0.06 | 4.51 | 0.21 | 2.89 | 0.07 |
| GI | 7.5 | C | 32.43 | 0.28 | 82.67 | 0 | 23.96 | 0.51 | 6.38 | 0.12 | 3.30 | 0.22 |
| $H_2O$ | 2.5 | T | 11.19 | 0.13 | 12.34 | 0 | 0.82 | 0.07 | 3.77 | 0.25 | 2.67 | 0.03 |
| $H_2O$ | 7.5 | T | 11.57 | 0.06 | 13.18 | 0.05 | 1.05 | 0.09 | 3.15 | 0.09 | 3.00 | 0.10 |
| GA | 2.5 | T | 30.67 | 0.24 | 36.52 | 0.09 | 1.19 | 0.03 | 5.81 | 0.11 | 2.95 | 0.10 |
| GI | 7.5 | T | 35.20 | 0.58 | 68.95 | 0.42 | 22.35 | 1.87 | 8.41 | 0.53 | 3.68 | 0.00 |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated

Table A.10. Yields of DPPH radical scavenging activity, metal chelation activity and total phenolic content of *in vitro* digests and $H_2O$ extracts of CWRS wheat bran[a]

| Extraction type | Time (hr) | Digest | Digest Constituents | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | DPPH (µmol TE/g bran) | | MCA ($Na_2EDTA$ µmol/g bran) | | TPC (µmol FAE/g bran) | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| $H_2O$ | 2.5 | C | 2.93 | 0.13 | 9.26 | 0.32 | 4.43 | 0.09 |
| $H_2O$ | 7.5 | C | 6.77 | 0.38 | 5.31 | 0.23 | 6.01 | 0.03 |
| GA-no pepsin | 2.5 | C | 17.51 | 0.87 | 21.14 | 0.34 | 5.06 | 0.30 |
| GI-no pancreatin | 7.5 | C | 28.92 | 0.19 | 26.66 | 1.02 | 10.45 | 0.21 |
| GA | 2.5 | C | 25.67 | 0.10 | 24.97 | 0.08 | 8.97 | 0.12 |
| GI | 7.5 | C | 35.84 | 0.26 | 34.65 | 1.15 | 19.08 | 0.40 |
| $H_2O$ | 2.5 | T | 11.31 | 0.01 | 10.53 | 0.59 | 5.18 | 0.10 |
| $H_2O$ | 7.5 | T | 8.90 | 0.16 | 11.08 | 0.55 | 4.21 | 0.19 |
| GA | 2.5 | T | 24.08 | 2.17 | 22.40 | 0.23 | 6.37 | 0.03 |
| GI | 7.5 | T | 37.16 | 1.16 | 33.32 | 1.26 | 16.53 | 0.08 |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated; DPPH, DPPH scavenging activity; MCA, Metal chelating activity; TPC, total phenolic content

Table A.11. Concentrations of ash, protein, starch, pentosan and β-glucan in *in vitro* digests of wheat brans[a]

| Bran | Treat | Digest | Digest constituents (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ash (%) | | Protein (%) | | Starch (%) | | Pentosan (%) | | β-glucan (%) | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | C | GA | 8.59 | 0.09 | 52.11 | 0.16 | 0.8 | 0.04 | 1.88 | 0.03 | 0.68 | 0.06 |
| | | GI | 5.14 | 0.00 | 37.05 | 0.06 | 22.1 | 0.65 | 1.18 | 0.02 | 0.41 | 0.01 |
| | T | GA | 9.81 | 0.10 | 40.36 | 0.11 | 1.4 | 0.00 | 2.57 | 0.17 | 0.98 | 0.01 |
| | | GI | 5.34 | 0.02 | 32.15 | 0.00 | 21.3 | 0.60 | 1.51 | 0.02 | 0.51 | 0.01 |
| CWRS | C | GA | 13.72 | 0.16 | 38.67 | 0.23 | 0.6 | 0.04 | 2.62 | 0.12 | 1.68 | 0.04 |
| | | GI | 10.13 | 0.09 | 33.34 | 0.00 | 9.7 | 0.21 | 2.57 | 0.05 | 1.33 | 0.09 |
| | T | GA | 16.08 | 0.13 | 25.19 | 0.06 | 0.8 | 0.02 | 4.00 | 0.08 | 2.04 | 0.07 |
| | | GI | 11.29 | 0.19 | 28.68 | 0.17 | 9.3 | 0.78 | 3.50 | 0.22 | 1.53 | 0.00 |
| CWSWS | C | GA | 11.26 | 0.11 | 46.34 | 0.06 | 0.5 | 0.04 | 3.21 | 0.11 | 1.48 | 0.00 |
| | | GI | 6.78 | 0.04 | 33.51 | 0.06 | 20.4 | 1.70 | 2.48 | 0.09 | 1.06 | 0.03 |
| | T | GA | 12.14 | 0.18 | 35.06 | 0.23 | 1.1 | 0.10 | 3.90 | 0.25 | 2.06 | 0.00 |
| | | GI | 6.53 | 0.08 | 27.48 | 0.06 | 20.7 | 2.02 | 2.88 | 0.23 | 1.27 | 0.02 |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated

Table A.12. Concentrations of DPPH radical scavenging activity, metal chelation activity and total phenolic content of *in vitro* digests of wheat brans[a]

| Bran | Treat | Digest | Digest constituents | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | DPPH (µmol TE/g) | | MCA ($Na_2EDTA$ µmol/g) | | TPC (µmol FAE/g) | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | C | GA | 105 | 3.41 | 116 | 10.24 | 38.9 | 1.07 |
| | | GI | 90 | 5.34 | 88 | 3.99 | 58.0 | 1.80 |
| | T | GA | 147 | 0.14 | 122 | 7.47 | 40.1 | 2.44 |
| | | GI | 108 | 9.05 | 101 | 7.26 | 49.5 | 2.64 |
| CWRS | C | GA | 149 | 0.60 | 145 | 0.45 | 52.1 | 0.70 |
| | | GI | 145 | 1.05 | 140 | 4.65 | 76.9 | 1.62 |
| | T | GA | 166 | 14.99 | 154 | 1.62 | 43.9 | 0.24 |
| | | GI | 155 | 4.82 | 139 | 5.24 | 68.7 | 0.35 |
| CWSWS | C | GA | 90 | 1.22 | 131 | 1.60 | 39.1 | 0.44 |
| | | GI | 85 | 3.88 | 111 | 4.73 | 56.9 | 1.86 |
| | T | GA | 114 | 3.35 | 133 | 0.55 | 31.7 | 2.28 |
| | | GI | 88 | 5.02 | 110 | 6.07 | 45.4 | 0.44 |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated; DPPH, DPPH scavenging activity; MCA, Metal chelating activity; TPC, total phenolic content

Table A.13. Yields of ash, protein, starch, pentosan and β-glucan in *in vitro* digests of wheat brans[a,b]

| Bran | Treat | Digest | Digest constituents | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ash (mg/g bran) | | Protein (mg/g bran) | | Starch (mg/g bran) | | Pentosan(mg/g bran) | | β-glucan (mg/g bran) | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | GA | C | 23.04 c | 0.23 | 114.95 c | 0.36 | 1.84 b | 0.10 | 4.14 c | 0.06 | 1.49 c | 0.12 |
| | GI | | 26.37 b | 0.02 | 153.65 a | 0.23 | 91.58 a | 2.68 | 4.91 b | 0.10 | 1.71 b | 0.05 |
| | GA | T | 22.50 d | 0.23 | 73.49 d | 0.20 | 2.59 b | 0.00 | 4.67 b | 0.31 | 1.79 b | 0.01 |
| | GI | | 27.92 a | 0.13 | 140.18 b | 0.00 | 92.74 a | 2.62 | 6.58 a | 0.08 | 2.21 a | 0.06 |
| CWRS | GA | C | 30.47 c | 0.36 | 66.56 c | 0.40 | 1.00 b | 0.06 | 4.51 c | 0.21 | 2.89 c | 0.07 |
| | GI | | 32.43 b | 0.28 | 82.67 a | 0.00 | 23.96 a | 0.51 | 6.38 b | 0.12 | 3.30 b | 0.22 |
| | GA | T | 30.67 c | 0.24 | 36.52 d | 0.09 | 1.19 b | 0.03 | 5.81 b | 0.11 | 2.95 bc | 0.10 |
| | GI | | 35.20 a | 0.58 | 68.95 b | 0.42 | 22.35 a | 1.87 | 8.41 a | 0.53 | 3.68 a | 0.00 |
| CWSWS | GA | C | 28.81 b | 0.27 | 94.22 c | 0.11 | 1.05 b | 0.09 | 6.53 c | 0.22 | 3.01 d | 0.01 |
| | GI | | 29.70 a | 0.19 | 118.51 a | 0.20 | 72.16 a | 6.01 | 8.76 b | 0.33 | 3.76 b | 0.11 |
| | GA | T | 25.23 c | 0.38 | 56.40 d | 0.37 | 1.84 b | 0.16 | 6.28 c | 0.40 | 3.32 c | 0.00 |
| | GI | | 30.19 a | 0.36 | 101.9 b | 0.21 | 76.00 a | 7.44 | 10.58 a | 0.84 | 4.68 a | 0.06 |

[a] Columns within bran types with different letters are significantly different ($P < 0.05$).
[b] GA, gastric; GI, gastrointestinal; C, control; T, treated

Table A.14. Yields of DPPH radical scavenging activity, metal chelation activity and total phenolic content of *in vitro* digests of wheat brans[a,b]

| Bran | Treat | Digest | Digest constituents | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | DPPH (μmol TE/g bran) | | MC (μmol $Na_2EDTA$/g bran) | | TPC (μmol FAE/g bran) | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | GA | C | 23.2 c | 0.75 | 25.6 c | 2.26 | 8.6 c | 0.24 |
| | GI | | 37.3 b | 2.21 | 36.4 b | 1.65 | 24.1 a | 0.75 |
| | GA | T | 26.7 c | 0.02 | 22.3 c | 1.36 | 7.3 c | 0.44 |
| | GI | | 47.2 a | 3.95 | 44.0 a | 3.17 | 21.6 b | 1.15 |
| CWRS | GA | C | 25.7 b | 0.10 | 25.0 b | 0.08 | 9.0 c | 0.12 |
| | GI | | 35.8 a | 0.26 | 34.7 a | 1.15 | 19.1 a | 0.40 |
| | GA | T | 24.1 b | 2.17 | 22.4 c | 0.23 | 6.4 c | 0.03 |
| | GI | | 37.2 a | 1.16 | 33.3 a | 1.26 | 16.5 b | 0.08 |
| CWSWS | GA | C | 18.2 b | 0.25 | 26.6 b | 0.33 | 8.0 c | 0.09 |
| | GI | | 30.1 a | 1.37 | 39.2 a | 1.67 | 20.1 a | 0.66 |
| | GA | T | 18.4 b | 0.54 | 21.5 c | 0.09 | 5.1 d | 0.37 |
| | GI | | 32.5 a | 1.85 | 40.5 a | 2.23 | 16.7 b | 0.16 |

[a] Columns within bran types with different letters are significantly different ($P < 0.05$).
[b] GA, gastric; GI, gastrointestinal; C, control; T, treated

Table A.15. Concentrations of Na, Ca, Cu, Fe, and K minerals in wheat brans and wheat bran *in vitro* digests[a,b]

| Bran | Treat | Sample | Digest Constituents (ppm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Na | | Ca | | Cu | | Fe | | K | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | C | Bran | 29.77 | 2.31 | 763.21 | 29.24 | 13.68 | 0.04 | 151.25 | 3.79 | 12064.07 | 653.38 |
| | C | GA | 46394.07 | 2006.78 | 115.67 | 3.41 | 25.27 | 0.01 | 21.53 | 0.72 | 27169.09 | 893.84 |
| | | GI | 26582.45 | 844.02 | 124.79 | 2.45 | 18.44 | 0.16 | 22.08 | 0.67 | 15815.58 | 195.46 |
| | T | GA | 55464.74 | 1773.35 | 158.58 | 8.11 | 23.51 | 0.02 | 34.94 | 1.97 | 32779.39 | 475.94 |
| | | GI | 25364.08 | 915.01 | 129.93 | 1.31 | 16.34 | 0.79 | 32.27 | 0.52 | 15112.54 | 159.16 |
| CWRS | C | Bran | 28.27 | 1.54 | 953.63 | 44.73 | 14.95 | 0.05 | 200.38 | 6.50 | 16735.92 | 616.18 |
| | T | Bran | 21.89 | 2.42 | 1011.86 | 63.17 | 15.59 | 0.18 | 210.51 | 10.75 | 17386.35 | 446.27 |
| | C | GA | 63496.24 | 2607.32 | 142.01 | 6.84 | 36.51 | 0.57 | 24.71 | 0.89 | 44983.49 | 2272.39 |
| | | GI | 48296.74 | 1664.68 | 141.34 | 4.68 | 29.18 | 0.31 | 37.37 | 1.39 | 35048.35 | 1227.17 |
| | T | GA | 72391.31 | 3271.16 | 168.57 | 7.88 | 28.22 | 0.02 | 38.72 | 0.51 | 51189.17 | 3065.37 |
| | | GI | 48832.68 | 1794.69 | 141.80 | 1.83 | 26.08 | 0.93 | 32.49 | 0.34 | 36009.41 | 509.39 |
| | C | GA - no pepsin | 72205.61 | - | 172.05 | - | 33.46 | - | 32.05 | - | 51156.17 | - |
| | C | GI - no pancreatin | 63974.43 | - | 130.86 | - | 33.51 | - | 41.77 | - | 43265.26 | - |
| CWSWS | C | Bran | 16.93 | 2.46 | 762.34 | 46.09 | 9.64 | 0.03 | 108.59 | 5.95 | 12455.82 | 211.57 |
| | C | GA | 52294.60 | 1555.18 | 121.87 | 5.22 | 24.37 | 0.09 | 17.40 | 0.80 | 36324.68 | 1503.60 |
| | | GI | 34372.57 | 1329.38 | 91.45 | 1.17 | 16.79 | 0.81 | 15.33 | 0.70 | 23848.84 | 497.93 |
| | T | GA | 62718.71 | 1982.47 | 152.60 | 4.75 | 20.27 | 0.08 | 28.37 | 0.87 | 44575.00 | 1024.02 |
| | | GI | 30763.31 | 1045.67 | 101.63 | 1.36 | 12.90 | 0.50 | 21.27 | 0.32 | 21321.13 | 152.09 |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated

[b] Single replicate analysis was performed for no pepsin and no pancreatin digests

Table A.16. Concentrations of Mg, Mn, P, S, and Zn minerals in wheat brans and *in vitro* wheat bran digests[a,b,c]

| Bran | Treat | Sample | Digest Constituents (ppm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mg | | Mn[a] | | P | | S | | Zn | |
| | | | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | C | Bran | 4953.25 | 44.75 | 143.63 | 5.92 | 11775.73 | 32.49 | 1991.43 | 34.06 | 116.59 | 7.79 |
| | C | GA | 2689.79 | 27.79 | 9.35 | 0.48 | 8754.69 | 110.66 | 4218.13 | 11.59 | - | - |
| | | GI | 2399.86 | 9.07 | 10.50 | 0.50 | 7766.82 | 14.67 | 2895.83 | 35.74 | - | - |
| | T | GA | 4128.54 | 66.14 | 15.70 | 0.95 | 11792.36 | 385.55 | 3258.38 | 42.91 | - | - |
| | | GI | 2565.65 | 22.13 | 13.20 | 0.42 | 7805.08 | 6.95 | 2324.79 | 6.77 | - | - |
| CWRS | C | Bran | 7610.25 | 158.72 | 231.34 | 11.35 | 18333.19 | 257.05 | 1611.95 | 35.28 | 141.98 | 10.56 |
| | T | Bran | 7984.20 | 319.94 | 243.01 | 15.67 | 19254.92 | 718.47 | 1708.83 | 41.05 | 150.93 | 11.45 |
| | C | GA | 3999.68 | 105.53 | 17.06 | 1.07 | 20029.80 | 751.12 | 3325.21 | 13.25 | - | - |
| | | GI | 3806.68 | 67.60 | 17.05 | 0.87 | 17791.30 | 470.34 | 2952.49 | 87.67 | - | - |
| | T | GA | 5052.48 | 2.66 | 22.38 | 0.73 | 23770.92 | 68.20 | 1976.31 | 4.47 | - | - |
| | | GI | 4524.53 | 1.17 | 19.99 | 0.59 | 19766.38 | 47.00 | 2203.35 | 4.40 | - | - |
| | C | GA - no pepsin | 4880.77 | - | 21.18 | - | 23448.64 | - | 2770.47 | - | - | - |
| | C | GI - no pancreatin | 3446.48 | - | 14.13 | - | 19171.62 | - | 3253.44 | - | - | - |
| CWSWS | C | Bran | 5198.33 | 157.02 | 104.92 | 7.23 | 11360.73 | 273.98 | 1915.47 | 0.16 | 70.48 | 3.66 |
| | C | GA | 3642.74 | 190.11 | 8.45 | 0.48 | 12591.65 | 263.28 | 4279.14 | 125.73 | - | - |
| | | GI | 2874.83 | 1.81 | 6.81 | 0.19 | 9958.51 | 59.15 | 3261.69 | 58.25 | - | - |
| | T | GA | 5052.61 | 27.42 | 12.42 | 0.51 | 15874.35 | 71.32 | 3278.63 | 4.57 | - | - |
| | | GI | 3194.31 | 11.49 | 8.67 | 0.21 | 10103.09 | 28.54 | 2375.61 | 55.44 | - | - |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated

[b] Single replicate analysis was performed for no pepsin and no pancreatin digests

[c] Zn values with "-" symbol was reported to be lower than 1 ppm

Table A.17. Yields of NaCl, Ca, Cu, Fe, and K in wheat brans and wheat bran *in vitro* digests[a,b]

| Bran | Treat | Digest | Extract Yield | Digest Constituents (mg/100 g bran) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NaCl | | Ca | | Cu | | Fe | | K | |
| | | | (g/100g bran) | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | C | GA | 26.81 | 3159.13 | 136.65 | 3.10 | 0.09 | 0.68 | 0.000 | 0.58 | 0.02 | 728.36 | 23.96 |
| | | GI | 51.32 | 3465.02 | 110.02 | 6.40 | 0.13 | 0.95 | 0.008 | 1.13 | 0.03 | 811.64 | 10.03 |
| | T | GA | 22.93 | 3230.00 | 103.27 | 3.64 | 0.19 | 0.54 | 0.001 | 0.80 | 0.05 | 751.54 | 10.91 |
| | | GI | 52.27 | 3367.71 | 121.49 | 6.79 | 0.07 | 0.85 | 0.041 | 1.69 | 0.03 | 789.99 | 8.32 |
| CWRS | C | GA | 22.21 | 3581.60 | 147.07 | 3.15 | 0.15 | 0.81 | 0.013 | 0.55 | 0.02 | 998.96 | 50.46 |
| | | GI | 32.03 | 3929.38 | 135.44 | 4.53 | 0.15 | 0.93 | 0.010 | 1.20 | 0.04 | 1122.64 | 39.31 |
| | T | GA | 19.07 | 3506.88 | 158.47 | 3.21 | 0.15 | 0.54 | 0.000 | 0.74 | 0.01 | 976.29 | 58.46 |
| | | GI | 31.18 | 3866.81 | 142.11 | 4.42 | 0.06 | 0.81 | 0.029 | 1.01 | 0.01 | 1122.60 | 15.88 |
| | C | GA - no pepsin | 18.28 | 3352.59 | - | 3.15 | - | 0.61 | - | 0.59 | - | 935.13 | - |
| | C | GI - no pancreatin | 23.72 | 3854.38 | - | 3.10 | - | 0.79 | - | 0.99 | - | 1026.25 | - |
| CWSWS | C | GA | 25.59 | 3399.12 | 101.09 | 3.12 | 0.13 | 0.62 | 0.002 | 0.45 | 0.02 | 929.56 | 38.48 |
| | | GI | 43.83 | 3826.80 | 148.00 | 4.01 | 0.05 | 0.74 | 0.036 | 0.67 | 0.03 | 1045.34 | 21.83 |
| | T | GA | 20.79 | 3311.48 | 104.67 | 3.17 | 0.10 | 0.42 | 0.002 | 0.59 | 0.02 | 926.58 | 21.29 |
| | | GI | 46.24 | 3612.90 | 122.81 | 4.70 | 0.06 | 0.60 | 0.023 | 0.98 | 0.01 | 985.82 | 7.03 |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated

[b] Single replicate analysis was performed for no pepsin and no pancreatin digests

[c] NaCl yield was determined by multiplying Na yield by the NaCl:Na ratio (1.54) since Cl was not determined

Table A.18. Yields of Mg, Mn, P, S, and Zn in wheat brans and wheat bran in *vitro* digests[a,b,c]

| Bran | Treat | Digest | Extract Yield | Digest Constituents (mg/100 g bran) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mg | | Mn | | P | | S | | Zn | |
| | | | (g/100g bran) | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| CWAD | C | GA | 26.81 | 72.11 | 0.74 | 0.25 | 0.013 | 234.70 | 2.97 | 113.08 | 0.31 | - | - |
| | | GI | 51.32 | 123.16 | 0.47 | 0.54 | 0.026 | 398.58 | 0.75 | 148.61 | 1.83 | - | - |
| | T | GA | 22.93 | 94.66 | 1.52 | 0.36 | 0.022 | 270.37 | 8.84 | 74.71 | 0.98 | - | - |
| | | GI | 52.27 | 134.12 | 1.16 | 0.69 | 0.022 | 408.00 | 0.36 | 121.53 | 0.35 | - | - |
| CWRS | C | GA | 22.21 | 88.82 | 2.34 | 0.38 | 0.024 | 444.81 | 16.68 | 73.84 | 0.29 | - | - |
| | | GI | 32.03 | 121.93 | 2.17 | 0.55 | 0.028 | 569.88 | 15.07 | 94.57 | 2.81 | - | - |
| | T | GA | 19.07 | 96.36 | 0.05 | 0.43 | 0.014 | 453.36 | 1.30 | 37.69 | 0.09 | - | - |
| | | GI | 31.18 | 141.05 | 0.04 | 0.62 | 0.019 | 616.22 | 1.47 | 68.69 | 0.14 | - | - |
| | C | GA - no pepsin | 18.28 | 89.22 | - | 0.39 | - | 428.64 | - | 50.64 | - | - | - |
| | C | GI - no pancreatin | 23.72 | 81.75 | - | 0.34 | - | 454.75 | - | 77.17 | - | - | - |
| CWSWS | C | GA | 25.59 | 93.22 | 4.86 | 0.22 | 0.012 | 322.22 | 6.74 | 109.50 | 3.22 | - | - |
| | | GI | 43.83 | 126.01 | 0.08 | 0.30 | 0.008 | 436.50 | 2.59 | 142.97 | 2.55 | - | - |
| | T | GA | 20.79 | 105.03 | 0.57 | 0.26 | 0.011 | 329.98 | 1.48 | 68.15 | 0.09 | - | - |
| | | GI | 46.24 | 147.69 | 0.53 | 0.40 | 0.010 | 467.14 | 1.32 | 109.84 | 2.56 | - | - |

[a] GA, gastric; GI, gastrointestinal; C, control; T, treated

[b] Single replicate analysis was performed for no pepsin and no pancreatin digests

[c] Zn values with "-" symbol was reported to be lower than 1 ppm