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Wheat Bran Dietary Fiber: Promising Source of Prebiotics with Antioxidant Potential

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Abstract

The potential of wheat bran (WB) addition as a prebiotic source were demonstrated using yogurt with probiotics (*Lactobacillus acidophilus* and *Bifidobacterium lactis*). Yogurts (with 4% WB) were significantly ($P < 0.05$) different in total bacterial counts (9.1 log CFU/mL), and total titratable acidity % (TTA, 1.4%) compared to controls during 28 days cold storage (4°C). Additionally, WB-total dietary fiber contents and their bound phenolic profiles were investigated as well as the antioxidant activity of WB-water extractable polysaccharides (WEP) was studied. HPLC analysis of alkaline hydrolyzed DF fractions showed that insoluble DF had higher phenolic acids (84.2%) content than soluble DF (15.8%). Also, crude-WEP showed stronger antioxidant activity compared to purified-WEP with an ORAC of 71.88 and 52.48 μmol TE/g, respectively. Here we demonstrate WB has potentials as a source of prebiotics, which may have the potentials for functional foods and nutraceutical applications.

Keywords: Bound phenolics, dietary fiber, DPPH, ORAC, prebiotics, wheat bran

1. Introduction

Increased whole grain consumption is linked to a decreased risk of chronic diseases (Fardet, 2010) such as obesity (Jonnalagadda et al., 2011), type II diabetes (Murtaugh et al., 2003), cardiovascular disease (Mellen, Walsh, & Herrington, 2008) and cancer (Schatzkin et al., 2008). Wheat (*Triticum aestivum)*, is second to rice as the main staple food crop. Most bioactive components (e.g. phenolic acids, alkylresorcinols) and dietary fiber (45%) are present in wheat bran (WB) fractions that represent 14-16% of the grain by weight (Fardet, 2010).

Cereal grain oligosaccharides act as prebiotics (a non-digestible food ingredient/soluble dietary fiber) and increase beneficial bacteria (probiotics) amount in the large bowel, thus improving gut health (Topping, 2007). They are a nutritional substrate for the probiotics in the colon and have the potential to improve host health (Chakraborti, 2011). Meanwhile phenolic compounds (e.g. ferulic and gallic acids) are related to prevention of diseases through some potential mechanisms such as free radical quenching, transition-metal chelation, and stimulation of the antioxidant enzyme system (Aaby, Skrede, & Wrolstad, 2005). Probiotics and prebiotics are commonly used in fermented dairy products (Kanmani et al., 2013) and supplementary research is desired to discover bio-products and their potential use in functional foods or nutraceuticals. Therefore, it was aimed to; 1) investigate the prebiotic effects of WB addition on microbial counts as colony forming units (CFU), and TTA in yogurts with and without probiotic bacteria, 2) measure the antioxidant activity of crude- and purified-WEP by oxygen radical absorbance capacity (ORAC), and 2, 2-diphenyl-1-picryhydrazyl (DPPH) assays along with total phenolic content (TPC), and 3) determine WB-total DF content (SDF, IDF) and analyze the phenolic acids and flavonoid composition of each fiber fraction as well as WB.

2. Materials and Methods

2.1 Materials

Analytical grade solvents including acetone, ethanol, methanol, ethyl ether, HCL, and ethyl acetate were purchased from Caledon Laboratories LTC (Georgetown, ON, Canada). Over 98% pure of fluorescein, mono-and dibasic potassium phosphate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2, 2'-azobis

(2-methylpropionamidine) dihydrochloride (AAPH), Folin- Ciocalteu (FC) reagent, α-tocopherol, and 2, 2-diphenyl-1-picryhydrazyl radical (DPPH), NaOH, sodium carbonate phenolphthalein were obtained from Sigma (Oakville, ON, Canada). Protease from *Bacillus licheniform* (saline solution ≥2.4 U/g protein, EC 232-560-9) and α-amylase from *Bacillus licheniformis* (Type XII-A, saline solution ≥500 U/mg protein, EC 232-752-2) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). The starter cultures, (*Lactobacillus delbrueckii* ssp. *bulgaricus* (B-548; USDA) and *Streptococcus salivarius* ssp. *thermophilus* (14485; ATCC)), probiotics (*Lactobacillus acidophilus* (B-4495, USDA) *and Bifidobacterium lactis* (41405, USDA)), Man Rogosa Sharpe (MRS) broth liquid and MRS agar media were purchased from Oxoid Ltd. (Basingstoke, United Kingdom).

Phenolic acids (gallic, protocatechuic, p-OH-benzoic, chlorogenic, caffeic, vanillic, syringic, p-coumaric, sinapic, ferulic, o-coumaric) and flavanoid standards (pyrogallol, catechin, epicatechin, rutin, quercetin-3-beta-glucoside, epicatechin gallate, myricetin, quercetin, apigenin and kaempherol) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

2.2 Prebiotic Activity

2.2.1 Sample Preparation

Wheat bran (WB) was kindly provided by Kraft Canada, freeze dried and kept in sealed plastic bags (-20°C) until further use. Before use, it was ground to 0.5 mm particle size at Agriculture-Canada (Ottawa, ON, Canada) Laboratories using a cyclone sample mill (UDY Corporation, CO, USA).

2.2.2 Milk Preparation

Pasteurized whole milk (3.25%), purchased from a local market, was heated at 85°C for 15 minutes, cooled down to 42°C in a water bath and transferred into 50 mL-sterile test tubes. The starter cultures, probiotics, and 4% WB were added and incubated at 42°C until the yogurt reached ~ pH 5.0 (Santo et al., 2010). All treatments were done in triplicates.

2.2.3 Microbial Cultures

The starter cultures (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*), probiotic 1 (*Lactobacillus acidophilus*) and probiotic 2 (*Bifidobacterium lactis*) were employed to make all yogurt trials as shown in Table 1. For each strain, sterile aliquots of MRS broth (10 mL) were used to grow microorganisms (incubated at 37°C for 24 h). For stock culture, activated tubes were used after three successive rinses with sterilized distilled water. Then the cultures were diluted with sterilized milk (121°C for 15 min in an autoclave) to obtain a concentration of 6.5 log bacteria cells/mL and then added to the tubes depending on the trials (Table 1) and the initial pH recorded. There were a total of eight yogurt trials including four with WB (4%) and four as controls without WB. Yogurts with WB were compared against corresponding controls. All tubes were incubated at 42 ℃ for fermentation and pH was measured after 4 h and 1 h thereafter. When the pH reached approximately 5, all tubes transferred were stored at 4 ℃ (Santo, et al., 2010).

Table 1. The experimental design used to evaluate the effect of WB addition on microbial counts in different yogurt trials

Yogurt trials	**Sample coding**
Y	Y
Y + Pro 1	Y+1
Y + Pro 2	Y+2
Y + Pro 1 & Pro 2	Y+1+2
Y + WB	YB
Y + WB + Pro 1	YB+1
Y + WB + Pro 2	YB+2
Y + WB + Pro 1 & Pro 2	YB+1+2

Y= standard yogurt containing only starter cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophiles*. Pro 1 = probiotic *Lactobacillus acidophilus*, Pro 2 = probiotic *Bifidobacterium lactis*, WB = wheat bran (4%). Equal volumes of each bacterium (6.5 log bacteria cells/mL) were added to the tubes depending on the treatments

2.2.4 Microbial Count

On days 1, 7, 14, 21 and 28, total microbial counts were carried in triplicate for each batch at different dilutions; four serial dilutions of 1 to 10. An aliquot (5 µL) from each dilution was plated on MRS agar dishes using a spread plate method and incubated at 37 ℃ for 24 h (Santo, et al., 2010).

2.2.5 Total Titratable Acidity (TTA) Measurements

Percent TTA for all yogurts were determined on the same days (1, 7, 14, 21 and 28 in triplicate) by titrating each yogurt sample with 0.1 N NaOH using 0.1% (w/v) phenolphthalein as an indicator. Additionally, pH of all yogurts were recorded on each day (Denver Instrument UB-5 pH meter) (Behrad, Yusof, Goh, & Baba, 2009).

2.3 Water Extractable Polysaccharides (WEB) -Antioxidant Activity

2.3.1 WEP-Extraction

Crude-WEP: WB and distilled water were mixed (1:100, w/v), stirred (70 ℃ for 4 h), cooled, centrifuged at 6000 x g for 20 min (Thermo Sorval, Legend XT Series, Fisher Scientific, Nepean, ON, Canada) and the supernatant was retained for further analysis.

Purified-WEP: Starch and proteins/peptides were removed by using α-amylase and protease to the supernatant solution (20 µL/100 mL) and agitated at 37 ℃ for 24 h. Then the supernatant was cooled and centrifuged (6000 x g for 20 min). The supernatant was dialyzed against double distilled water for 48 h and replaced with fresh distilled water every 6 h to separate polysaccharides and other materials with a molecular weight cut-off of 3500 Da (Spectra/Por, CA, USA). The extract was kept at -20 ℃ until further analysis (Escarnot et al., 2011).

2.3.2 Total Phenolic Content (TPC)

Crude- and purified-WEP total phenolic contents were measured by the Folin-Ciocalteu (FC) method (Gao, Wang, Oomah, & Mazza, 2002). Each sample extract of 200 µL was mixed with FC reagent (1.9 mL of 10-fold diluted FC), and then 1.9 mL of sodium carbonate solution (60 g/L) was added to the mixture. All tubes were stored in the dark at room temperature for 2 h, and the absorbance values were recorded at 725 nm (UV-Vis. Spectrophotometer, Cary 50 Bio, Varian Inc., Australia) against a blank of distilled water. Ferulic acid (FA) was employed as standard, and TPC values were calculated as FA equivalents per gram of samples (Gunenc, HadiNezhad, et al., 2013).

2.3.3 DPPH Assay

For DPPH radical scavenging activity of crude- and purified-WEP, Brand-Williams et al. (1995) study was followed. Each sample extract (200 µL) was reacted with DPPH solution (3.8 mL) for 1 h, and then absorbance (A) values of the mixture at 515 nm were read against a blank of pure 95% ethanol. The antioxidant activity was calculated as % discoloration (Gunenc, Tavakoli, et al., 2013; Li et al., 2009).

2.3.4 Oxygen Radical Absorbance Capacity (ORAC) Assay

ORAC values of crude- and purified-WEP were determined by a fluorometric micro plate reader (FLx800™ Multi-Detection Micro plate Reader with Gen5™ software, BioTek Instruments, Ottawa, Canada) described by Gunenc et al., (2013). All analyses were carried out at 37 ℃ with a 20 min incubation and 60 min run-time. A micro plate was prepared containing 20 µL of Trolox standards, sample dilutions, as well as 120 µL of fluorescein (FL) solution. After the incubation of the plate, 60 µL of 153 mM AAPH was added quickly to each well for a final volume of 200 µL. FL micro plate reader was used to read the absorbance during run-time (at an excitation wavelength of 485 nm and an emission wavelength of 525 nm). ORAC values were calculated by the differences of net areas under the FL decay curves between the blank and sample and were expressed as micromole Trolox Equivalents per gram of sample (µmol TE/g) (Gunenc et al., 2015; Ou et al., 2002).

2.4 Total Dietary Fiber (TDF) and Its Phenolics

2.4.1 Extraction of TDF

WB-Total dietary fiber as the sum of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) was calculated by following the AOAC Official Method (991.43). To remove starch and protein, WB (5 g) was exposed to enzymatic digestions; firstly, heat stable α-amylase (250 µL, boiling water bath for 30 min), then alcalase protease (50 mg/mL, 500 µL, pH 7.5, 60°C for 30 min) and lastly, amyloglucosidase (1500 µL, pH 4.5, 60˚C for 30 min). After centrifugation (10, 000 rpm), the residue was washed sequentially with hot water, ethanol (95%), and acetone (95%); filtered and recorded as IDF.

The combined supernatants from the washings was precipitated in ethanol (80%, preheated to 60˚C, 4 volumes) overnight and recorded as SDF. The two fractions (IDF and SDF) were placed in a fume hood and dried at

35-40°C overnight to remove organic solvent (Guo & Beta, 2013).

2.4.2 Phenolic Compound Extractions from WB, SDF and IDF

WB, SDF and IDF were alkaline hydrolyzed to release their bound phenolics and followed by liquid-liquid partitioning steps to extract those released phenolic compounds (Gunenc, et al., 2015; Guo & Beta, 2013). More specifically, each sample (WB, SDF, and IDF) were mixed with 2 M NaOH and agitated at room temperature for 4 h. Then, they were acidified (6M HCl) to pH 1.5-2 and liquid-liquid partitioning steps were followed. The dried alkaline extracts were re-dissolved in MeOH and filtered (0.45 μm PTFE) before HPLC analysis (Kim, Tsao, Yang, & Cui, 2006).

2.4.3 HPLC Analysis of Phenolic Compounds from WB, SDF and IDF

The extracts from section 2.4.2 were analyzed via a reverse phase (RP)-HPLC on the Alliance® HPLC system e2695, Separation Module with the 2998 PDA (Waters, Mildford, Massachusetts, USA) with Empower 3 software. All phenolic acid and flavonoids standards as well as the extracts were prepared in methanol. To separate phenolic acids and flavonoids in a single run with a Synergy-Max-RP column, at 35°C, solvent-A (0.01% formic acid:Milli-Q water) and solvent-B (100% acetonitrile) at a flow rate of 1.0 mL/min and a linear gradient program from 90% to 50% solvent A in 35 min was developed. Phenolic acids (280 nm) and flavonoids (320 nm) were identified and quantified using 11 phenolic acids and 10 flavonoid standards (Gunenc, et al., 2015).

2.5 Statistical Analysis

Statistical analysis was performed using Analysis of variance (ANOVA) with Statistical Analysis System (9.2, SAS Institute Inc., Cary, NC). All experiments were accompanied in triplicates. Duncan's Multiple Range test was used when significant ($P < 0.05$) mean comparison was achieved.

3. Results and Discussion

3.1 Prebiotic Activity

3.1.1 Microbial Count (log CFU/mL)

Total microbial counts on days 1, 7, 14, 21 and 28 of fermentations in all yogurt trials were shown in Figure 1. By day 1, microbial counts increased to a range of 8.19-8.31 log CFU/mL in control yogurts, and 8.41-8.65 log CFU/mL in yogurts with WB from an initial bacteria count of 6.5.

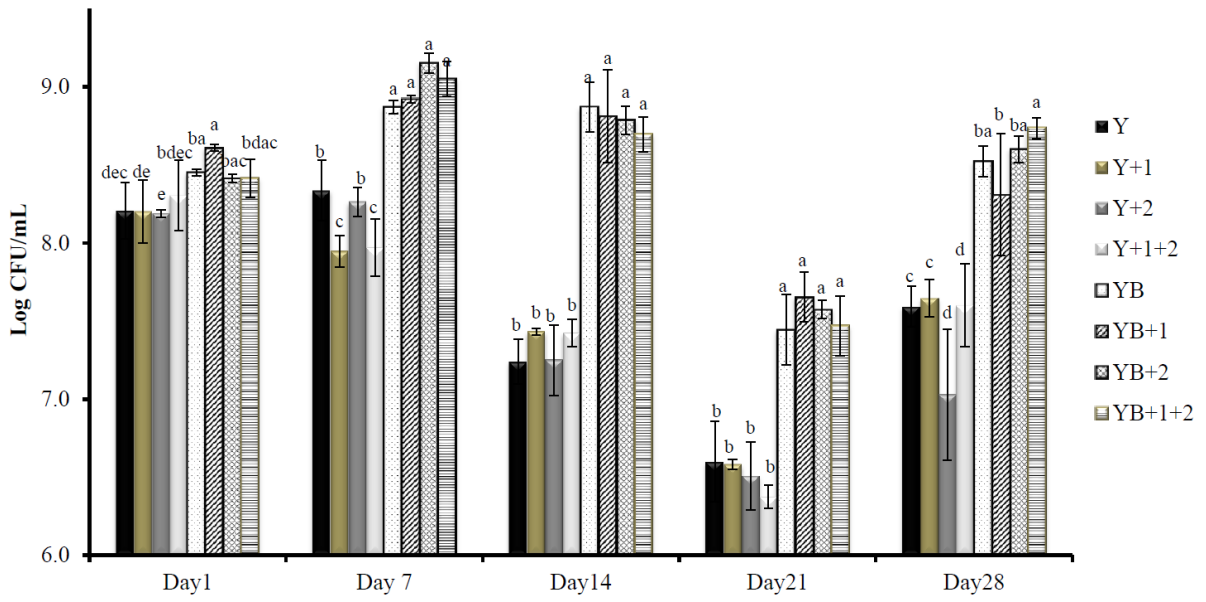

Figure 1. Total microbial count (log CFU /mL) in control yogurts (Y, Y+1, Y+2, Y+1+2) and yogurts with 4% wheat bran (YB, YB+1, YB+2, YB+1+2). Different letters in columns on the same day are significantly different ($P<0.05$)

By day 7, the bacterial growth increased significantly ($P < 0.05$) in yogurts with WB (8.87 and 9.15 log CFU/mL) compared to their corresponding controls (7.94 to 8.34 log CFU/mL). By days 14 and 21, the CFU values in yogurt samples with and without WB continued to be significantly different.

By day 28, yogurt samples lacking WB had significantly ($P < 0.05$) lower total bacteria counts of 7.03-7.65 log CFU/mL compared to 8.31-8.74 log CFU/mL of yogurt samples with WB. It has been suggested that the amount of viable bacteria remaining in yogurt after four weeks of cold storage should be in the range of 6 to 8 log CFU/mL (Vasiljevic & Shah, 2008). The controls remained within the range, but the yogurts containing WB had higher bacteria counts in the range of 8.31 and 8.74 log CFU/mL.

Overall, the yogurts with WB verified significantly higher bacteria counts ($P < 0.05$) during the four weeks of cold storage in comparison to control samples. It can be interpreted that in the presence of WB, there was an increase in microbial viability in sample trials consisting of probiotics. The randomized human study of Fran çois et al. (2012) showed that addition of 3g/day WB increased bifidobacteria (1.3 fold) and significantly increased faecal propionic acid levels (Francois et al., 2012). It may be a synergistic, additive or antagonistic effect of WB on probiotics compared to its corresponding controls. These results showed that WB might have a selective effect on increasing probiotics and starters during cold storage from day 1 to day 14, and to a lesser extent on day 21. A decreasing trend in CFU numbers may be a result of nutrient depletion over time or their metabolites (Sengun, Nielsen, Karapinar, & Jakobsen, 2009). For all microbes, certain nutrients such as iron and manganese are necessary to promote viability and growth. At the same time, probiotic bacteria have the ability to bind these elements and reduce accessibility by pathogenic bacteria (Bomba, Nemcová, Mudronová, & Guba, 2002). WB consists of manganese, and iron, with levels of 4-14 and 2.5-19 g/100g (Fardet, 2010).

Additionally, the Food and Drug Administration (FDA) has issued a letter of no objection for the WB extract Brana Vita, giving manufacturers a new prebiotic for a range of food and beverage products (Daniells, 2010). So, yogurts with WB and probiotics might supply micronutrients and oligosaccharides, selectively stimulating these particular bacterial strains (Costa, Queiroz-Monici, Machado-Reis, & Oliveira, 2006).

3.1.2 TTA %

During four weeks storage, TTA of all yogurt trials (Figure 2) with WB additions (YB, YB +1, YB +2, YB +1 +2) showed increasing TTA %, indicating that lactic acid production was increased as a result of the growing number of bacteria. The control yogurts had significantly lower TTA% than corresponding yogurts with WB. On day 28, the yogurt treatment with both probiotics (YB +1 +2) had the highest value from their corresponding control yogurt (Y +1 +2). It might be due to secondary metabolites production (lactate, propionate, or ethanol) and their positive influence on cell viability (Rattanachaikunsopon & Phumkhachorn, 2010). Moreover, volatile aroma compounds like carbonyl compounds might increase cell sustainability without impacting pH levels (Beshkova et al., 2003). The significant drop in pH on day 28 did not seem to intrude cell viability. Also parallel findings has been reported for improved probiotic viability in yogurt samples with low pH levels (4.1) in the presence of a prebiotic source (Agil & Hosseinian, 2012; Santo, et al., 2010).

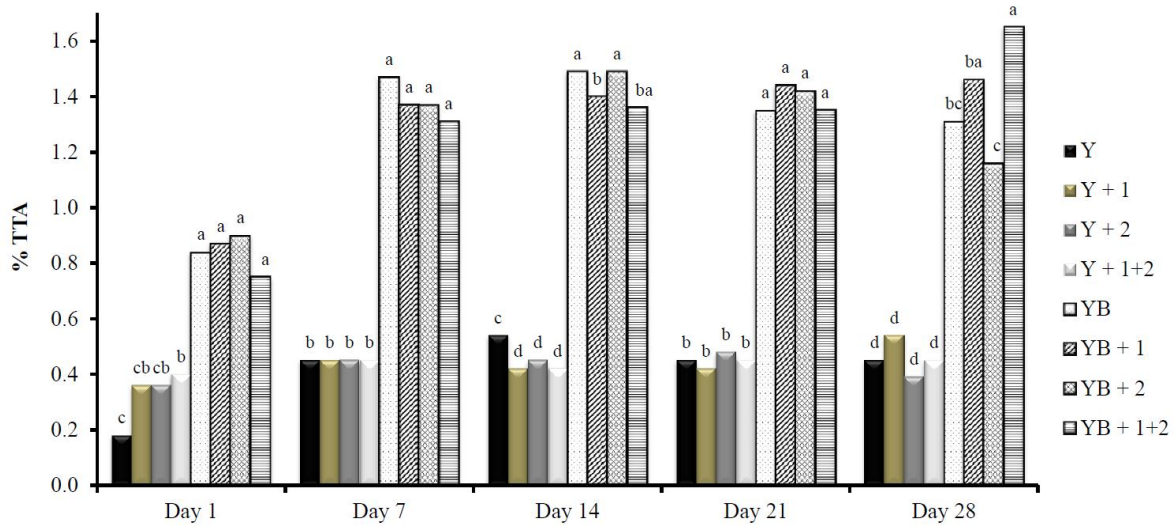

Figure 2. Percent total titratable acidity (%TTA) in control yogurt (Y, Y+1, Y+2, Y+1+2) and yogurt with 4% wheat bran (YB, YB+1, YB+2, YB+1+2). Different letters in columns on the same days are significantly different (P<0.05). TTA % values in and. Number 1 and 2 represent probiotic1 and 2. These results, parallel to our findings, suggest that the addition of WB to yogurts caused bacteria to produce more lactic acid, confirming findings obtained from the pH monitoring

3.2 Antioxidant activity of WEPs

3.2.1 ORAC Assay

The ORAC value (Table 2) of crude-WEP (71.88 ± 4.01 µmol TE/g) was higher than purified-WEP (52.48 ± 2.05 µmol TE/g). Moore et al. (2006) reported ORAC values of 45 to 78 µmol TE/g for 20 wheat bran samples (Moore, Liu, Zhou, & Yu, 2006). Also in another study, ORAC values of six wheat ranged from 19.5 to 37.4 µmole TE/g (Okarter, Liu, Sorrells, & Liu, 2010). Our ORAC findings are higher than above mentioned studies. This might be due to different wheat varieties, particle size, and some differences in the experimental procedures. Also, the antioxidant activity could be attributed to the presence of bound phenolic acids in the crude extract (e.g. ferulic and p-coumaric acids) (Hosseinian & Mazza, 2009). Moreover, presence of sugars with acyl groups and/or glycan-polymerization has been reported to have effects on the antioxidant activity of polysaccharides (Rao & Muralikrishna, 2006).

3.2.2 DPPH Assay

The %DPPH of crude- and purified-WEP were 9.65 ± 0.17 and 4.16 ± 0.18, respectively (Table 2). Similar to the study of Hromádková et al (2013), crude-WEP showed high antioxidant activity in both DPPH and ORAC tests (Hromádková et al., 2013). Phenolic components such as phenolic acids have been described to have a significant role in the overall radical scavenging capacity of xylans and xylooligosaccharides from the WB (Veenashri & Muralikrishna, 2011).

Table 2. ORAC, DPPH and TPC values of WEP

Antioxidant assays	**Purified WEP**	**Crude WEP**
ORAC (µmole TE/g)	52.48 ± 2.05^{b}	71.88 ± 4.01^{a}
%DPPH	4.16 ± 0.18^{b}	9.65 ± 0.17^{a}
TPC (mg FAE/g)	2.96 ± 0.10^{b}	6.18 ± 0.84^{a}

ORAC = Oxygen radical absorbance capacity as µmole Trolox Equivalent (TE)/g of sample.

DPPH (%) = 2, 2-Dipheny-1-picryhydrazyl radical (DPPH) radical scavenging activity assay

TPC = Total phenolic count was calculated as mg ferulic acid equivalent (FAE)/g of sample

WEP = Water extractable polysaccharides and values are means of triplicates ± standard deviation (SD)

Different letters in rows are significantly different (P < 0.05) in Duncan's multiple range tests

3.2.3 TPC

TPC of crude-WEP was over twice that of purified-WEP analyzed by FC method (6.18 ± 0.84 vs 2.96 ± 0.10 mg FAE/g) (Table 2) reflecting its higher antioxidant activity. The purification process probably removed considerable amount of phenolic compounds such as phenolic acids, flavonoids, phenolic acid diacyl glycerols, phenolic aldehydes and ferulates (Fardet, 2010). The most ample phenolic acid is FA followed by di-FA, sinapic acid, p-coumaric acid, caffeic acid and benzoic acid derivatives (Adom & Liu, 2002). About 95% of phenolic compounds in cereal grains are linked to cell wall polysaccharides by ester bonds and classified as dietary fiber-phenolic compounds (Hatfield, Ralph, & Grabber, 1999). Consequently, the addition of WB in yogurt might affect the gastrointestinal tract by acting like potential prebiotics, improving probiotic viability and functioning as antioxidants, especially after colonic fermentation (Fardet, 2010).

3.3 TDF and its Phenolics

3.3.1 TDF

TDF content (53%) was counted as a sum of SDF (6%) and IDF (47%). The content and proportion of both DF fractions are diverse among different types of cereals. This might be due to differences in seed morphology among most cereal grains (including wheat, maize and barley). Most of them have higher IDF than SDF, with WB dietary fiber around 44.5% (Fardet, 2010). Also, the DF content of WB (containing both outer layers and germ) and WB (only outer layers) were found 52.2% and 59.3%, respectively (Frolich & Asp, 1981). Our results are in close range with the above studies and literature (Stevenson, Phillips, O'Sullivan, & Walton, 2012).

3.3.2 Phenolic Compounds from WB, SDF and IDF

Table 3 shows the bound phenolic acid and flavonoid contents (mg/g of sample) of WB, IDF, and SDF. All three samples (WB, SDF, and IDF) were hydrolyzed prior to liquid-liquid extraction of bound phenolic acids. WB phenolic acid content (2.64 mg/g) was higher compared to that of total dietary fiber (IDF+SDF; 1.46 mg/g). This was an expected result since each step of fractional DF extraction resulted in some phenolic acid loss (Guo & Beta, 2013). IDF had higher total phenolic acid content compared to SDF fraction.

Table 3. HPLC profiles of bound-phenolic compounds in the fractions of both soluble/insoluble dietary fibers and WB

Phenolics	**SDF***	**IDF**	**WB**
Gallic	0.10^{a}	0.15^{b}	0.24^{c}
Proto-catechuic	nd	0.06^{cb}	nd
p-OH-benzoic	0.03^{c}	0.08^{cb}	0.17^{d}
Chlorogenic	nd	nd	0.89^{b}
Vanillic	0.01^{cd}	nd	0.04^{e}
Syringic	0.01^{cd}	0.03^{d}	0.05^{e}
p-coumaric	0.02^{cd}	0.07^{cb}	nd
Sinapic	nd	nd	0.11^{de}
Ferulic	0.06^{b}	0.84^{a}	1.13^{a}
Total phenolic acids (PA)	**0.23 ± 0.02**	**1.23 ± 0.01**	**2.64 ± 0.25**
Catechin	0.01^{cd}	0.07^{cb}	0.10^{de}
Rutin	0.03^{c}	0.07^{cb}	0.13^{de}
Quercetin-3-beta glucoside	0.03^{c}	0.08^{cb}	nd
Epicatechin gallate	nd	nd	0.12^{de}
Total Flavonoid (FC)	**0.07 ± 0.01**	**0.22 ± 0.09**	**0.35 ± 0.12**
Total phenolics (PA+FC)	**0.30 ± 0.02**	**1.45 ± 0.05**	**2.99 ± 0.17**

*Values are means of triplicates, and different letters in columns are significantly different ($P < 0.05$) in Duncan's multiple range tests. SDF = Soluble dietary fiber fraction, IDF = Insoluble dietary fiber fractions, WB = wheat bran, nd = not detected.

Most total content of phenolic compounds (PA + FC) in both IDF and SDF fractions were found in insoluble fractions as in our previous study (Gunenc, et al., 2015). For example, 84.24% PA, 75.86% FC and 82.86% of total phenolic compounds were found in IDF fractions.

Nine phenolic acids including gallic, protocatechuic, p-OH-benzoic, chlorogenic, vanillic, syringic, p-coumaric, sinapic and ferulic acids were determined in HPLC analyses of WB, IDF, and SDF. Ferulic acid was the predominant phenolic acid, and mainly found as bound form in IDF (93.3%). Also, gallic acid was predominant in SDF. Our findings are in parallel with the study of Guo and Beta (2013).

With HPLC, Four flavonoid peaks were identified and respectively assigned as catechin, rutin, quercetin-3-beta glucoside, and epicatechin gallate (Table 3). IDF had more flavonoids (0.22 mg/g) than SDF (0.07 mg/g). Those biologically active components have not received much consideration as the phytochemicals in fruits and vegetables although the increased consumption of whole grain products has been linked to a diminished risk of chronic diseases (Liu, 2007). Our flavonoid content findings were in the same range reported by Singh, Sharma and Sarkar (2012) and close range with the study of Feng and McDonald (1989) in which the mean flavonoid content of four wheat classes were characterized and reported to be 0.29 mg/g, and our corresponding flavonoid content of total DF and WB were 0.29 and 0.35 mg/g respectively (Feng & Mc Donald, 1989; Singh, Sharma, & Sarkar, 2012).

4. Conclusions

This study showed that WB enhanced bacterial survival and growth in yogurt over 28 days cold storage period at 4 ℃. The overall increase in TTA% values in yogurts containing WB during the storage period suggest that WB could be consumed by probiotics. Furthermore, crude-WEP showed stronger antioxidants activity than purified-WEP as well as IDF has higher phenolic content compared to SDF. Consequently, DF can act as a carrier of phenolic compounds. It can be concluded that WB might be used as a potential source of prebiotics with higher antioxidant activity for functional foods and nutraceutical applications. Further investigations are needed for nutritive values (mineral solubility) and sensory evaluation of yogurt samples with WB addition.

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