Consumption of whole purple and regular wheat modestly improves metabolic markers in adults with elevated high-sensitivity C-reactive protein: a randomised, single-blind parallel-arm study

Tamer H. Gamel[1,2], El-Sayed M. Abdel-Aal[2], Amy J. Tucker[1], Shannon M. Pare[1], Kate Faughnan[1], Charlene D. O'Brien[1], Andrea Dykun[1], Iwona Rabalski[2], Mark Pickard[3] and Amanda J. Wright[1]*

[1]*Department of Human Health & Nutritional Sciences, College of Biological Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada*
[2]*Guelph Research & Development Centre, Agriculture and Agri-Food Canada, Guelph, Ontario N1G 5C9, Canada*
[3]*Infra-Ready Products Ltd, Saskatoon, Saskatchewan S7M 5T2, Canada*

Abstract

Whole-grain wheat, in particular coloured varieties, may have health benefits in adults with chronic metabolic disease risk factors. Twenty-nine overweight and obese adults with chronic inflammation (high-sensitivity C-reactive protein) > 1·0 mg/l) replaced four daily servings of refined grain food products with bran-enriched purple or regular whole-wheat convenience bars (approximately 41–45 g fibre, daily) for 8 weeks in a randomised, single-blind parallel-arm study where body weight was maintained. Anthropometrics, blood markers of inflammation, oxidative stress, and lipaemia and metabolites of anthocyanins and phenolic acids were compared at days 1, 29 and 57 using repeated-measures ANOVA within groups and ANCOVA between groups at day 57, with day 1 as a covariate. A significant reduction in IL-6 and increase in adiponectin were observed within the purple wheat (PW) group. TNF-α was lowered in both groups and ferulic acid concentration increased in the regular wheat (RW) group. Comparing between wheats, only plasma TNF-α and glucose differed significantly ($P < 0.05$), that is, TNF-α and glucose decreased with RW and PW, respectively. Consumption of PW or RW products showed potential to improve plasma markers of inflammation and oxidative stress in participants with evidence of chronic inflammation, with modest differences observed based on type of wheat.

Key words: Whole wheat and bran: Inflammation: Oxidative stress: Anthocyanins: Phenolic acids: Overweight and obesity

Whole-grain wheat consumption is associated with reduced risk of CVD and type 2 diabetes[1–3], largely attributed to the bran fraction which is rich in fibres, phenolic acids, antioxidants, S-containing amino acids, vitamins and minerals[4–6]. As reviewed previously[7], foods based on whole-grain wheat can attenuate markers of oxidative stress, as well as inflammation. Studies have shown improvements in inflammatory markers, including C-reactive protein (CRP)[8] and TNF-α, when whole-grain wheat was the only[9] or the predominant[10,11] grain in the diet, although other studies have shown no effect[12]. The contributions of specific grain constituents to the observed risk reduction effects of whole grains remain unresolved. Phenolic acids and their metabolites contribute by serving as free radical scavengers, reducing agents and quenchers of singlet oxygen formation that inactivate reactive oxygen species[13,14]. Dietary fibres contribute by modulating glycaemic response, lowering blood cholesterol and increasing faecal bulk, subject to fibre type[15,16]. Other phytochemicals

(e.g. anthocyanins and flavonoids) present in whole grains[17], including coloured varieties, may confer additional health benefits.

Phytochemicals (e.g. anthocyanins, flavonoids, phytates, lectins), some of which contribute colour to grains, may interfere with nutrient digestion and absorption (e.g. phytic acid leading to Zn deficiency)[18]. Plant breeding and processing have sought to decrease phytochemical concentrations because of their anti-nutritional activities or to increase them based on health-enhancing potential, since many molecules can have positive or negative consequences, depending on the context (e.g. molecule, dose, degree of processing, bioavailability, animal species, health conditions)[19]. For example, polyphenols, and in particular the phenolic acids, ferulic and p-coumaric acids, negatively influence energy utilisation in sorghum-based broiler diets[20] by binding proteins and inhibiting digestive enzymes, but a higher intake of flavonoids in humans has been associated with lower incidence of type 2 diabetes[21]. As such, there is

---

* **Corresponding author:** Amanda J. Wright, email ajwright@uoguelph.ca

interest in teasing out the potential of phytochemicals that may be health-promoting (for example, anthocyanins) and incorporating these into emerging grain varieties. This includes colourful specialty whole-grain wheat varieties[22,23], one example of which is purple wheat (PW). This relatively new grain is rich in anthocyanins, especially cyanidin-3-glucoside[23]. PW and its fractions, including the bran, can be incorporated as ingredients into a range of functional food products[24]. However, most research with cereal anthocyanins is limited to *in vitro* and animal models[25]. Mrkvicova *et al.*[26] observed some changes in antioxidant activity and liver enzyme activities in rats, chickens and fish who were fed PW. Human studies of coloured grain foods and coloured wheat and PW, specifically, are scarce and mostly limited to extracted pigmented fractions. For example, Wang *et al.*[27] showed that black-pigmented rice fraction improved cardiovascular risk factors, including high-sensitivity C-reactive protein (hs-CRP). Liu *et al.*[28] conducted a 5-week intervention where participants with type 2 diabetes consumed products (steamed buns, noodles, and peeled kernels mixed with rice and mung beans) containing a relatively new variety of anthocyanin-rich black-grained wheat or control regular white and rice cereal products, with all participants given nutritional and diabetes education. Participants in the black-grained wheat group had a decrease in glycated albumin. Also, TNF-α which tends to increase in type 2 diabetes did not increase for participants consuming the black-grained wheat, but did for the control group.

Our previous work showed that PW bars inhibited *in vitro* radical activity by 76 % according to the oxygen radical absorbance capacity method[29]. It also confirmed that, when healthy adults consumed PW bars and crackers, the anthocyanins were rapidly absorbed and excreted, with no short-term impacts on plasma antioxidant activity or the inflammatory markers IL-6 and TNF-α[30]. In general, the majority of anthocyanin studies are limited to fruits and vegetables, as thoroughly reviewed[31,32]. Although this evidence is strong and suggests mechanisms for anti-obesity effects, and reducing oxidative stress and inflammation[31], the data cannot be fully extrapolated to coloured grains. The objective of the present study was to explore if 8-week consumption of products based on whole PW, compared with whole regular wheat (RW), with weight maintenance, would alter markers of inflammation, oxidative stress and lipaemia in overweight or obese individuals with chronic inflammation. It was hypothesised that consumption of both whole-grain products would positively influence metabolic markers, but that, because of its anthocyanin content, PW would have a greater influence.

Methods

Study materials and products

Whole-grain PW (CDC Primepurple cultivar, commercially distributed by InfraReady Products Ltd, Saskatoon, SK as AnthoGrain™) and RW (CDC Utmost cultivar) were used to produce bran-enriched convenience bars by an automated process at the Saskatchewan Food Industry Development Centre

Inc., Saskatoon, SK., following good manufacturing and sanitary practices. PW was developed based on the CDC Utmost cultivar and owes its namesake colour to the enrichment in anthocyanin pigments. In each case, a blend of 50 g whole wheat flakes and 50 g wheat bran was combined with maize syrup, sugar, rapeseed oil and xanthan gum (30, 10, 10 and 0·5 g, respectively). The dry ingredients were mixed with the maize syrup, compressed, shaped into thick sheets, cut into bars and dried at 300°F for 30 min. Products were shipped frozen to the University of Guelph, immediately portioned into baggies in 40 g servings, sealed from air and moisture, packaged in fours to provide the daily serving amount (160 g), labelled, and placed in frozen storage (–18 ± 2 °C).

Product analysis

Nutrient analysis was performed after milling the bars to fine particles using an M2 universal mill (IKA-Werke). Protein (N × 5·7) was measured by the combustion method using a nitrogen analyser (FP 2000 Leco Instrument UK Ltd.). Moisture, starch, ash, crude fat and total dietary fibre were determined according to American Association of Cereal Chemists International Approved Methods 44–40.01, 76–13.01, 08–01.01, 30–20.01 and 32–05.01, respectively[33]. Anthocyanins were quantified after extraction with acidified ethanol[23]. Free and bound phenolic acids were separated and quantified following extraction with 80 % methanol to obtain free phenolic acids followed by saponification to liberate bound phenolic acids[30,34]. The anthocyanins and phenolic acids were separated and quantified[22] using an 1100 series chromatograph (Agilent Tech.) and malvinidin-3-glucoside as an internal standard. Anthocyanins were detected at 525 nm and phenolic acids at 260, 275 and 320 nm using nine anthocyanin standards (>97 % purity) (Polyphenols AS), and six phenolic acids purchased from Sigma-Aldrich Co.

Human study

Trial design and participant enrolment. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Research Ethics Board of the University of Guelph (16MR006). Written informed consent was obtained from all subjects/patients. The clinical trial was registered (NCT02840357) at ClinicalTrials.gov (https://clinicaltrials.gov/ct2/show/NCT02840357?term=02840357&draw=2&rank=1).

A randomised, single-blind parallel-arm study was conducted at the Human Nutraceutical Research Unit, at the University of Guelph, Ontario from June 2016 to September 2017. The trial ended when all recruited eligible participants finished the intervention and in order to end the research activities within the funding agency timelines.

Participants were recruited from Guelph, Ontario, and the surrounding area through various channels. The study inclusion criteria were age 18–70 years; BMI ≥ 25 kg/m² and/or waist circumference ≥102 cm (men) and ≥88 cm (women); serum hs-CRP over 1·0 mg/l (based on the American Heart Association[35]); stable (>3 months) and consistent use of any prescribed medications

and/or supplements; non- to moderate alcohol consumption; not taking antibiotics within the last 3 months or planning to take antibiotics within the next 6 months. Exclusion criteria were smoking, pregnancy or breast-feeding, lifetime history of any acute medical event, including but not limited to, heart attack or stroke; recent diagnosis (within 6 months) of a serious medical condition; any disorder of the gastrointestinal system or food intolerances, any food allergies; and use of anthocyanin-specific supplements. Eligibility was assessed through a phone-screening questionnaire and in-person screening visit where a fasted blood sample was collected by venepuncture for determination of the study primary outcome hs-CRP by an accredited medical laboratory (LifeLabs, Ontario, Canada). All eligible and interested participants provided written informed consent. A sample size calculation (OpenEpi, version 3, open source calculator; http://www.openepi.com/SampleSize/SSMean.htm) using CI (95 %), power (80 %) and SD of 20 % indicated that thirty-two participants (sixteen per group) would be required, based on a 20 % change in CRP in one group compared with the other, as estimated in previous studies, that is, Lefevre & Jonnalagadda[36] for a whole-grain intervention and Zhu et al.[37] for an anthocyanin supplement intervention.

Study intervention and procedures. Participants were assigned randomly following stratified block randomisation procedures (computer-generated list) to one of two groups; bran-enriched whole PW (intervention) or bran-enriched whole RW (control) convenience bars, aiming for balanced group sizes and equal distribution of male and female participants between groups. Participants were enrolled into the study after approval of the principal investigator and assigned to treatment group by the lead study coordinator. The PW and control bars differed in colour, with the PW products being darker, but not obviously purple, such that participants could not know which treatment group they were allocated to. To maintain blinding, study coordinators other than the lead coordinator distributed coded products to participants throughout the intervention. Data were also re-coded from participant number to help obscure treatment allocation throughout analysis by the lead coordinator and others. Participants were instructed to limit their consumption of high anthocyanin/phenolic content foods (e.g. coffee, berries, wine, black tea) to a maximum of two servings per d for a 10-d run-in period and throughout the intervention. They were asked to replace four daily servings of grain food products with the four servings (160 g total, based on Canada's Food Guide guidelines)[38] of whole-wheat bars for 8 weeks. Participants attended study visits on days 1, 29 and 57 and three additional check-in visits to ensure compliance to study protocols and to receive study products. Two days prior to each study visit, participants were requested to avoid consuming all high anthocyanin/phenolic acid-containing foods, alcoholic beverages and over-the-counter medications and supplements (unless prescribed by a healthcare professional) and to refrain from participating in strenuous physical activity. For 24 h prior to each study visits, participants were asked to adhere to their typical diet and to consume the same foods, supported by providing participants a copy of their food intake from their first visit. Participants were

also asked to observe a 12- h overnight fast where no intake of food or drink was permitted, except water, prior to each study visit. For premenopausal female participants, study visits were scheduled within the proliferative phase (approximately days 6–13 of the menstrual cycle). At days 1, 29 and 57, participants provided fasted venous blood samples and had body weight, height, waist circumference and blood pressure determined[30] and received study products. Participants completed gastrointestinal questionnaires to track the incidence of any symptoms (i.e. abdominal discomfort, bloating, cramping, rumbling, flatulence and bowel movement number, urgency and consistency) in the 24 h prior to each visit and provided 3-d food records for 2 week days and 1 weekend day in the week prior to each study visit. Participants were instructed to store the wheat bars frozen and to consume the daily portion periodically throughout each day, in place of other grain servings. Compliance was monitored based on returned empty bags and unconsumed bars and using a daily study diary that also captured details (e.g. illness, injury, new medication/supplement use), which were discussed with a study coordinator.

Collection and analysis of blood samples. On days 1, 29 and 57, fasting blood samples were collected into BD Vacutainer blood tubes by an approved phlebotomist through an antecubital vein in the arm. For determination of hs-CRP, a blood sample (6 ml) was collected in an SST tube with no anti-coagulant, allowed to clot at room temperature for 30 min and serum separated by centrifugation at 2600 rpm (572 g at mean r) for 10 min at 4°C. Blood was otherwise collected in heparin-coated tubes and centrifuged at 3000 rpm (665 g at mean r) for 10 min at 4°C. Plasma was sent directly to a medical laboratory for analysis, as below, or aliquoted and stored at –80°C, until analysis. For the anthocyanin and phenolic acid metabolite analyses, plasma was acidified with 50 % formic (50 µl/1 ml plasma) prior to –80°C storage in light-sensitive polypropylene tubes.

Fasting plasma TAG, total cholesterol, LDL- and HDL-cholesterol, glucose and insulin concentrations and serum hs-CRP were determined by Lifelabs. The homeostatic model assessment of insulin resistance (HOMA-IR 2) was calculated using the online calculator https://www.dtu.ox.ac./homacalculator/uk according to Levy et al.[39]. Commercial kits were used to determine plasma levels of oxidised LDL (hydroxynonenal) (Ox-LDL, Cell Biolabs Inc., no. STA-389), adiponectin (Affymetrix eBioscience, no. BMS2032) and glutathione (GSH, Cayman Chemical, no. 703002). IL-4, IL-6, IL-10 and TNF-α were determined using Invitrogen ELISA kits (Thermo Fisher Scientific Inc., no. EH3IL4, KHC0062 and EH3TNFA, respectively). IL-10 data were not analysed since 80 % of sample values fell below the kit's limit of detection (LOD). Intra- and inter-assay CV were between 2·4–3·7 % and 2–4·5 %, respectively. Plasma samples were analysed for anthocyanins, phenolic acids and metabolites by ultra-high-pressure liquid chromatography-MS (UPLC-MS, Waters Acquity UPLC H-Class with ACQUITY column manager and ACQUITY-Photometric detector), as previously described[30] and with a cyanidin-3-glucoside detection limit of 5 ng/10 µl injected volume.

Data and statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics for Windows, version 25.0.: IBM Corp. (2017) and $P < 0.05$ significance level. Data are presented as mean values and standard deviations, unless otherwise indicated. Before analysis, extreme outliers (> mean ± 3-fold of SD) were eliminated[40]. This included a single value of TNF-α and the complete dataset for one participant who had extreme glucose and lipid concentrations. Where values of plasma hs-CRP (1 % of values), IL-4 (20 % of values) and the metabolites (12 % of values) below the assays' detectable limits, these data points were replaced by one half of the LOD (LOD/2)[41].

The assumption of homogeneity of variances between the two groups was verified prior to each ANOVA using Levene's test, and the assumption of sphericity was verified in all cases of repeated-measures ANOVA. Unless otherwise stated, these assumptions were valid. Where homogeneity of variances was violated, data were log-transformed, and, where sphericity was violated, the Greenhouse–Geisser correction was applied. Independent t tests were used to determine differences in each parameter between the two treatments at baseline (day 1). ANCOVA was used to determine differences between the treatments at day 57 using day 1 as a covariate. Repeated-measures ANOVAs were used to evaluate the effect of days in the pooled dataset and within each treatment group, separately, as well the overall interaction of days × treatment. Specifically, when days was significant in the model, the Bonferroni adjustment was applied to assess differences between pairs of days 1, 29 and 57.

Results

Product characteristics

The nutrient and polyphenol compositions of the bars are presented in Table 1. The daily portion of four servings of the PW bars provided 1·65 mg anthocyanins. As expected, there were no anthocyanins detected in the RW bars. On the other hand, the whole PW and RW bars provided similar amounts of phenolic acids (i.e. 215 and 190 mg/4 servings, respectively), with ferulic being the dominant species in both. Both bars were also enriched in total dietary fibre because of the inclusion of bran. They contained similar levels of soluble fibre, although the PW bars were significantly higher (approximately 2 g/d) in insoluble fibre. Comparable bar caloric energy and available carbohydrate compositions were prioritised and successfully achieved.

Participant study diaries

The CONSORT flow diagram (Fig. 1) shows that thirty-three participants were randomised to either the PW (n 17) or RW (n 16) groups. Daily study diaries indicated that participants were compliant in limiting anthocyanin-rich foods and beverages and regularly consumed the bars, that is, overall rates of bar consumption were 99·4 and 97·3 % for PW and RW participants, respectively. According to food diaries kept for three 3-d periods, preceding days 1, 29 and 57, there were no significant differences between the groups for any period. Table 2 shows the pooled analysis of nutrient intakes. There was an effect of the intervention regardless of treatment group, whereby, over

Table 1. Nutrient, anthocyanin and phenolic acid contents of whole purple and whole regular wheat convenience bars*
(Mean values and standard deviations, wet weight basis, n 3)

	Convenience bar study treatments			
	Whole purple wheat		Whole regular wheat	
	Mean	SD	Mean	SD
Nutrient (g/100 g)				
Moisture	11·3[a]	0·5	15·5[b]	0·2
Protein	6·0[a]	0·1	7·3[a]	0·6
Crude fats	7·0[a]	0·9	8·5[a]	1·0
Starch	35·7[a]	1·4	34·4[a]	0·5
Ash	2·1[a]	0·1	1·9[a]	0·1
Total dietary fibre†	27·8[a] (44·5 g)	0·4	25·3[b] (40·5 g)	0·5
Insoluble fibre†	25·8[a]	0·1	23·7[b]	0·8
Soluble fibre†	2·0[a]	0·1	1·6[a]	0·3
Energy (kcal)†‡	502		498	
Anthocyanins (µg/g)	10·2 (1650 µg)	1·3	Not detected	
Phenolic acids, µg/g				
Free phenolic acids (FPA)	270[a]	17·0	210[b]	2·0
Bound phenolic acids (BPA)	1075[a]	52·0	970[a]	35·0
Total FPA + BPA	1345[a] (215 mg)	35·0	1180[b] (190 mg)	36·0
Protocatechuic acid	130[a]	2	256[b]	2
Caffeic acid	160[a]	6	93[b]	12
p-Coumaric acid	50[a]	4	100[b]	7
Ferulic acid	484[a]	21	331[b]	13
Sinapic acid	191[a]	3	154[b]	5
Unidentified acids	330[a]	8	246[b]	9

[a,b] Within a row, values with unlike superscript letters are significantly different ($P < 0.05$).
* Total amount in the daily 4–40 g servings is indicated in parentheses.
† Energy content calculated based on proximate analysis.
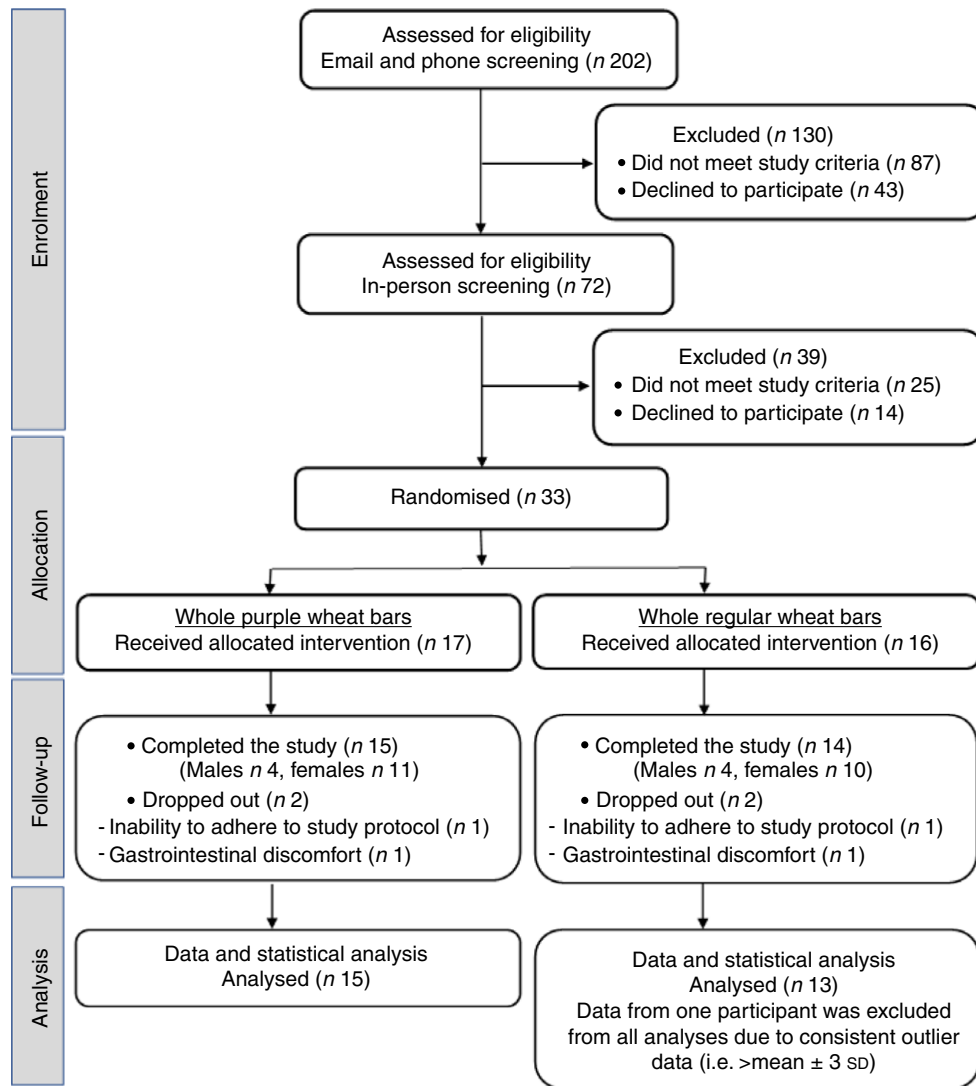‡ To convert energy values from kcal to kJ, multiply by 4·184.

Fig. 1. CONSORT participant flow diagram.

Table 2. Average daily energy and nutrient intakes based on 3-d food records preceding days 1 (baseline), 29 and 57
(Mean values and standard deviations, n 29)

	Pre-intervention		Mid-intervention		Post-intervention		
	Mean	SD	Mean	SD	Mean	SD	P*
Energy (kcal)†	2038	483	2017	576	2071	571	0·83
Carbohydrate (g)	240[a]	66	276[b]	68	274[b]	63	0·001
Dietary fibre (g)	21·6[a]	7·9	57·4[b]	7·5	56·6[b]	9·4	<0·001
Total sugars (g)	87·2[a]	30·4	111·6[b]	34·0	114·1[b]	31·8	<0·001
Protein (g)	92·4	27·7	79·8	24·0	87·0	23·5	0·08
Fat (g)	78·3	26·5	74·0	29·0	76·8	33·9	0·70
Saturated fat (g)	27·2[a]	11·3	22·1[b]	11·0	22·3[b]	11·6	0·01
Cholesterol (mg)	273	176	225	150	257	158	0·17

[a,b] Within each row, values with unlike superscript letters are significantly different (P < 0·05).
* Repeated-measures ANOVA P comparing between days.
† To convert energy values from kcal to kJ, multiply by 4·184.

time, average intakes of total carbohydrates, total dietary fibre and total sugars increased, and average intakes of saturated fat and protein intakes decreased. Intakes were consistent over time for energy, total fat and cholesterol (Table 2). Participants consumed an average daily energy intake of 8544 (SD 2272) kJ (2042 (SD 543) kcal) across the entire study duration, which is in line with a healthy energy intake for this demographic.

Retrospective gastrointestinal questionnaires were completed for three 24-h periods throughout the study and revealed that, although there were no differences in symptoms between the groups pre-intervention or between PW and RW ($P > 0.05$; data not shown), both interventions significantly increased participant perception of stomach rumbling ($P = 0.05$), flatulence ($P = 0.02$) and bowel movement urgency ($P < 0.001$) and number ($P < 0.001$), with improvements in bowel movement consistency ($P = 0.04$), over time.

Baseline (day 1) and treatment × day interactions

There were no observed baseline (day 1) differences between the PW and RW groups for any parameter (Table 3). Furthermore, there were no treatment × day interaction effects for any parameter investigated (Table 4).

Anthropometrics, plasma glucose, insulin and lipids

After 8-week consumption, only plasma glucose decreased significantly in PW compared with RW ($P = 0.04$). There were no differences between the PW and RW groups for anthropometrics, insulin, adiponectin or lipids (Table 3). BMI was not significantly different between or within groups, and from day 1 to 57 average BMI values were 30·7 (SD 3·7) to 30·9 (SD 3·6) kg/m^2 with PW, and 27·2 (SD 4·5) to 26·8 (SD 4·9) kg/m^2 with RW ($P > 0.05$). HOMA-IR was also unchanged within each group and between groups ($P > 0.05$). Average calculated values for HOMA-IR for day 1 and day 57, respectively, were 1·7 (SD 0·9) to 1·8 (SD 0·8) with PW, and 1·7 (SD 0·7) to 2·0 (SD 1·0) with RW. None of these parameters were significantly different in the pooled participants (data not shown).

Inflammatory markers

Within the PW group, adiponectin increased 23 % ($P = 0.01$) from days 1 to 57 (Table 3). Also, within the PW group only, IL-6 decreased significantly ($P = 0.04$) at 8 weeks and there was a trend for a reduction in TNF-α ($P = 0.06$; Table 3). Within the RW group, positive effects on TNF-α were observed at 4 and 8 weeks, with significant reductions ($P = 0.003$) observed at both time points. In the pooled participants, both adiponectin and TNF-α were significantly different over time (Table 4).

Participant serum hs-CRP levels were not significantly affected by either PW or RW consumption. The only difference in inflammatory markers observed between the groups was in TNF-α ($P = 0.02$; Table 3).

Oxidative stress markers

Plasma antioxidant activity was assessed by tracking changes in the oxidative stress markers GSH and ox-LDL. Within each group, and between groups, there were no changes in either parameter (Table 3). Within the pooled data (n 29), a significant 33 % increase in plasma GSH level was observed after 8 weeks (Table 4).

Plasma anthocyanins and phenolic acid metabolites

No parent anthocyanins were detected in the plasma of any participants, after 4 or 8 weeks of consumption of PW bars that contributed 1·65 mg of daily anthocyanins. Six phenolic acid metabolites, including ferulic and hippuric acids, were detected in both groups at each time point. After 8 weeks, there was a 2-fold increase ($P = 0.04$) in ferulic acid with RW and plasma ferulic acid also increased in the pooled data ($P = 0.02$; Table 4), but this was unchanged with PW and no changes were observed for hippuric acid. There also were no differences between the PW and RW groups in terms of total phenolic acids, ferulic acid or hippuric acid (Table 3).

Discussion

This is the first human intervention study to investigate the effects of consuming whole PW and RW in individuals with evidence of chronic inflammation, and only the second human study of PW to our knowledge. The study had a food replacement protocol with a run-in period and diet controls, food and energy intake records, and monitoring of gastrointestinal symptoms. Participants in both groups maintained their body weight, suggesting they were successful in substituting the daily 2092 kJ (500 kcal) serving of bars for other servings of refined grains or snack foods. Participant groups were equivalent at baseline for all outcome measures. By including individuals over a wide age range (25–69 years) and with overweight and obese BMI (25·3–56·9 kg/m^2), the generalisability of the results to the general North American population is relatively high. The inclusion of individuals with stable medical conditions, so long as medication use was consistent, was also a pragmatic decision. Overall, the study participants possessed different CVD risk factors, although all had evidence of low-grade chronic inflammation. At baseline, participant serum hs-CRP levels ranged from 1·03 to 10·84 mg/l (average of 3·3 (SD 2·5) mg/l), that is, levels associated with moderate to high risk of CVD[35]. Roughly 50 % of the study participants had two CVD risk factors according to the American Heart Association classifications, and 20 and 30 % had three and four, respectively.

Plasma analysis did not indicate the presence of intact anthocyanins in participants' blood. This may be attributed to PW's low anthocyanin content, low absorption and/or rapid metabolism. Our previous study also did not detect any anthocyanins or main metabolites in the plasma of healthy individuals within 8 h of consumption of PW bars and crackers which contained 6·7 mg anthocyanin, although there was evidence of urinary excretion (18–22 ng/ml)[30]. In the present work, it was hypothesised that, in spite of the low anthocyanin level in the PW products, with regular consumption, accumulation and cumulative physiological effects related to intact anthocyanins or their metabolites would occur. Plasma anthocyanin accumulation was not observed, but there was evidence of a cumulative physiological benefit of PW, as below. The relatively low level of anthocyanins in the PW product is a limitation of the study, as is the high dose of wheat products (i.e. four grain servings). Moreover, both products contained high levels of dietary fibre and this may have partly driven the metabolic effects observed. To

Table 3. Outcome measures at days 1 (baseline), 29 and 57 for participants in the purple and regular wheat groups*
(Mean values and standard deviations)

	Purple wheat							Regular wheat							
	Day 1		Day 29		Day 57			Day 1		Day 29		Day 57			
	Mean	SD	Mean	SD	Mean	SD	P†	Mean	SD	Mean	SD	Mean	SD	P†	ANCOVA P‡
Body weight (kg)§	85·9	16·0	90·3	21·4	86·8	15·8	0·36	97·4	22·2	97·9	22·6	98·3	22·7	0·11	0·99
Systolic BP (mmHg)§	119	16	125	12	122	15	0·42	120	11	120	13	119	11	0·93	0·31
Diastolic BP (mmHg)§	73	7	79	15	78	6	0·19	76	10·0	77	7	75	9	0·69	0·05
Glucose (mmol/l)	5·6	0·4	ND		5·4	0·4	–	5·3	0·4	ND		5·4	0·4	–	0·04
Insulin (pmol/l)	90·4	48·3	ND		95·0	45·8	–	92·2	37·7	ND		107·2	53·8	–	0·44
TAG (mmol/l)	1·22	0·56	1·34	0·42	1·47	0·67	0·20	1·51	0·55	1·49	0·64	1·52	0·62	0·97	0·42
Total cholesterol (mmol/l)‖	5·09	0·87	5·02	0·77	5·04	1·00	0·91	5·00	0·76	4·94	0·93	4·88	0·84	0·53	0·65
HDL-cholesterol (mmol/l)	1·51	0·28	1·48	0·32	1·48	0·29	0·83	1·38	0·37	1·40	0·35	1·37	0·29	0·88	0·83
LDL-cholesterol (mmol/l)	3·03	0·72	2·93	0·74	2·90	0·92	0·88	2·94	0·65	2·86	0·76	2·81	0·63	0·31	0·96
Ox-LDL (mg/l)	999	222	983	351	932	311	0·33	1052	246	1123	362	1047	426	0·56	0·57
hs-CRP (mg/l)‖	3·4	2·5	2·9	2·8	3·3	3·2	0·19	3·2	2·6	4·0	3·3	3·2	2·7	0·28	0·95
IL-4 (ng/l)§	12·5	10·1	9·5	9·9	9·4	9·1	0·23	7·2	5·8	8·2	9·4	11·1	10·8	0·18	0·15
IL-6 (ng/l)§‖	4·0[a]	2·5	3·2[a]	2·0	3·0[a]	1·8	0·04¶	4·1	1·9	4·5	1·9	3·4	1·4	0·29	0·39
TNF-α (ng/l)	30·5	10·7	28·0	8·9	27·0	9·4	0·06	29·6[a]	13·9	23·4[b]	10·1	22·6[b]	8·2	0·003	0·02
GSH (μmol/l)	10·0	5·2	9·9	4·7	12·5	3·8	0·08	7·2	3·0	9·4	5·4	10·6	5·0	0·11	0·53
Adiponectin (mg/l)‖	11·6[b]	5·0	13·0[a,b]	5·9	14·3[a]	6·6	0·01	14·0	4·3	15·8	7·2	15·1	4·9	0·22	0·16
Total phenolic acids (ng/ml)‖	1726	1437	1690	1277	1733	1096	0·99	1025	787	1146	820	1182	893	0·37	0·71
Ferulic acid (ng/ml)‖**	89	132	103	155	103	116	0·35	68[a]	87	106[a]	135	157[a]	179	0·04¶	0·50
Hippuric acid (ng/ml)‖	392	476	458	497	438	426	0·38	288	248	257	207	297	226	0·58	0·56

BP, blood pressure; ND, not determined; Ox-LDL, oxidised LDL; hs-CRP, high-sensitivity C-reactive protein.
[a,b] Unlike superscript letters indicate significant difference ($P < 0.05$) between days within each group.
* Mean of data for group participants and standard deviations (n 15 for purple wheat, except for TNF-α where n 14; n 13 for regular wheat group).
† Repeated-measures ANOVA P comparing between days within each wheat category.
‡ Significant difference in ANCOVA between purple and regular wheat groups at day 57, treating day 1 as a covariate ($P < 0.05$).
§ In the purple wheat group, the assumption of sphericity was violated so the Greenhouse–Geisser correction was applied.
‖ In the regular wheat group, the assumption of sphericity was violated so the Greenhouse–Geisser correction was applied.
¶ Although the overall difference between days was significant ($P < 0.05$), pairwise *post hoc* comparisons were not statistically different.
** Assumption of homogeneity was violated at day 57, treating day 1 as a covariate, so data were log-transformed prior to analysis. Arithmetic means are shown.

Table 4. Pooled participant data at days 1 (baseline), 29 and 57*
(Mean values and standard deviations)

Measures	Day 1 Mean	SD	Day 29 Mean	SD	Day 57 Mean	SD	P† between days	P‡ (days × treatment interaction)
Ox-LDL (mg/l)	1024	230	1048	357	986	367	0·36	0·59
hs-CRP (mg/l)	3·3	2·5	3·4	3·0	3·3	3·0	0·38	0·13
IL-4 (ng/l)	9·8	8·9	8·6	9·8	10·0	10·0	0·55	0·06
IL-6 (ng/l)§	4·1	2·2	3·8	2·4	3·2	1·6	0·08	0·26
TNF-α (ng/l)	30·0[a]	12·1	25·7[b]	9·6	24·9[b]	9·0	0·000	0·23
GSH (μmol/l)	8·7[b]	4·5	9·7[a,b]	4·9	11·6[a]	4·4	0·02	0·46
Adiponectin (mg/l)	12·7[b]	4·8	14·4[a,b]	6·6	14·7[a]	5·8	0·01	0·26
Total phenolic acids (ng/ml)	1420	1196	1466	1080	1477	1027	0·90	0·80
Ferulic acid (ng/ml)§	70[a]	97	88[a]	107	128[a]	148	0·02	0·30
Hippuric acid (ng/ml)§	356	378	386	393	372	349	0·73	0·30

Ox-LDL, oxidised LDL; hs-CRP, high-sensitivity C-reactive protein.
[a,b] Unlike superscript letters indicate significant difference ($P < 0.05$) between days.
* Mean of pooled data for all participants and standard deviations (n 28, except for TNF-α where n 27).
† Repeated-measures ANOVA P comparing between days.
‡ Significant day × treatment interaction ($P < 0.05$).
§ Assumption of sphericity was violated, so the Greenhouse–Geisser correction was applied.

enrich the PW bars in anthocyanins to the highest extent possible, bran was used, resulting in participants in both groups consuming >40 g dietary fibre, daily. This serving of PW bars was previously well tolerated by healthy male and female study participants who consumed them in one setting[30]. In the present study, one participant dropped out from each group, related to gastrointestinal upset. Indeed, there were some perceived gastrointestinal symptoms and overall improvements in bowel movement consistency over time in both groups. Future functional food developments could explore de-branning methods that further enrich bran anthocyanin content[42] in order to minimise the required serving size and should also consider effects of food matrix[29] and processing[43]. As it stands, the present study adds to the evidence that high-fibre food products based on whole-grain wheat can shift the metabolic profile in at risk adults, without reductions in body weight when a replacement strategy is used.

The main metabolites detected in participants' plasma with RW and PW consumption were ferulic and hippuric acids. Ferulic acid is the main phenolic acid found in wheat and especially of the bran fraction[44]. Hippuric acid is a urine and plasma metabolite seen after coffee consumption[45]. Dietary records thoroughly assessed for non-compliance did not indicate that observations of hippuric acid were associated with particular foods or beverages. Interpersonal variation based on genetic and/or background diet could be important factors influencing intervention impact. Prospective screening of plasma for detection of relevant compounds should be considered in human studies, as should faecal analysis, recognising that anthocyanins can alter and their metabolism can be altered by gut microbiota[46]. There was some evidence of phenolic acid accumulation, that is, plasma ferulic acid increased in the pooled participants after 8 weeks, driven by RW consumption ($P = 0.02$; Table 3). Vitaglione et al.[9] reported that consumption of 70 g/d of 100 % whole-grain shredded wheat biscuits for 4–8 weeks by overweight and obese individuals resulted in a 4-fold increase in serum dihydroferulic acid and a 2-fold increase in faecal ferulic acid compared with refined wheat crackers or toasted bread, which showed no changes. In the present study, PW had higher levels of bound and total (free + bound) phenolic acids than RW. More work needs to be done to understand the metabolism of anthocyanins and phenolic acids derived from PW and should include analysis of other biological samples, for example, faeces, urine, breath.

Importantly, our findings support a role for whole-grain wheat interventions to improve plasma TNF-α after a 4-week period with significant decreases in the pooled data and RW group and a trend to decrease within the PW group ($P = 0.06$). Similarly, Vitaglione et al. (2015) observed significant reductions in plasma TNF-α after consumption of 70 g of whole-grain v. refined wheat products daily for 8 weeks by overweight and obese participants who maintained a habitual diet devoid of whole-grain cereals and cereal bran-containing products, low fruit and vegetables intake and a low level of physical activity[9]. Price et al.[12] compared the effects of a high-wheat aleurone diet (27 g aleurone/d) v. control in healthy, older, overweight participants (n 79) for 4 weeks and found no changes in terms of inflammatory markers studied or plasma antioxidant status. The ability to modify plasma TNF-α within 4 weeks, along with participant weight maintenance, is a strength of this intervention.

Whole-grain interventions of wheat are limited but have suggested a role in protecting circulating adiponectin levels compared with refined grains[47]. In the present study, improvements in plasma adiponectin were seen in the pooled data and adiponectin and IL-6 improved in the PW group. The preservation of adiponectin in RW and increase in PW, while body weight was maintained during a high-carbohydrate diet, reveals a benefit of the whole-wheat high-fibre intervention. That said, differences in hs-CRP were not observed. While human studies of PW, specifically, do not exist, there have been investigations of anthocyanin-rich supplements, as reviewed by meta-analysis. Comparing seventeen human intervention trials of anthocyanin-rich supplements from mostly fruits (average of >200 mg/d), the evidence favours anthocyanin intake for improving HDL-cholesterol, LDL-cholesterol, apo A1, apo B, TAG and TNF-α, with no benefit on total cholesterol, IL-6 or hs-CRP, compared with

placebo[48]. Therefore, our results showing a PW-specific effect on IL-6 and adiponectin are unique and may be attributed to the whole-grain wheat intervention. Grain-derived anthocyanin supplements have been studied, previously. For example, 6-month supplementation with 10 g daily black rice fraction powder significantly reduced hs-CRP (1·26 mg/l change), as well as plasma total antioxidant capacity and CVD risk factors in patients (n 30) with CHD[27]. The present study saw no changes in hs-CRP, potentially because it was underpowered, with high inter-participant variability in a parallel-arm design, and used a relatively low dose of anthocyanins. However, the present results are in line with a cross-sectional study of 938 healthy men and women showing that whole-grain intake was not associated with reductions in the inflammatory markers CRP and IL-6[49], and with an intervention study by Andersson et al.[50] that found that daily consumption of 112 g whole-grain products by 30 healthy moderately overweight adults for two 6-week periods did not affect hs-CRP or IL-6. Hoevenaars et al.[8] showed that 12-week whole-grain wheat consumption lowered fasting CRP compared with refined wheat, in fifty overweight and obese participants with hypercholesterolaemia and noted the challenges with observing improvement in very healthy participants. While whole-grain consumption has been associated with lower hs-CRP levels in older women and individuals with certain health conditions[51], this is not supported by all studies, including the present one. hs-CRP plays an important diagnostic role but is not specific to only chronic inflammation and is therefore a challenging study marker. The value of measuring various circulating inflammatory markers in intervention studies is evident.

According to the pooled analysis, consumption of the RW and PW products improved plasma oxidative status. A high correlation was recently reported between antioxidant activity of coloured wheat genotypes, blue wheat and PW, and their contents of total phenolics and anthocyanins[52], with PW flagged as a promising functional food ingredient. There have been many in vitro and several in vivo studies that support a role for cereal anthocyanins to improve antioxidant capacity, many of which have been thoroughly reviewed[25]. Most in vitro studies have been conducted using ingredients or laboratory prepared food products. Previously, our team assessed the acute plasma and urine bioavailability and plasma antioxidant capacities of PW bars and crackers in healthy adults and showed no effect on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) or 1,1-diphenyl-2-picrylhydrazyl radical scavenging activities[30]. In the present study, levels of GSH did not increase significantly in the PW ($P = 0.08$) or RW ($P = 0.11$) groups but did increase in the pooled dataset ($P = 0.02$), suggesting a cumulative effect of whole wheat on this key intracellular thiol that regulates cell redox status[53], reduces cellular damage from oxidative stress and prevents development of CVD[54]. This agrees with other whole-grain intervention studies showing improvements related to antioxidant activity of wheat bran phenolics[55–57].

There were no changes in insulin or HOMA-IR in the PW, RW or pooled groups. This is consistent with a previous 6-week whole-grain wheat-based diet intervention study in healthy overweight adults[50] and 12-week whole-grain wheat intervention in overweight and obese adults with hypercholesterolaemia[8].

However, fasting glucose was significantly lowered with consumption of the PW v. RW ($P = 0.04$). This result is in agreement with a human study where black-grained wheat positively influenced glycated albumin and certain markers of inflammation v. non-coloured control foods in adults with type 2 diabetes[28]. It is also consistent with in vitro evidence that anthocyanins from purple rice bran increased glucose uptake and inhibited the activity of the starch digestion enzyme glucosidase[58]. Therefore, the PW intervention showed potential to improve glycaemic management that was not seen in the RW or pooled data, that is, beyond a whole-grain wheat effect. Aside from the low level of anthocyanins present in the PW products, these contained higher levels of insoluble fibre (approximately 2 g/d, but the same level of soluble fibre) suggesting colonic fermentation may have contributed to the augmented differences, although this requires further study.

Conclusion

Consumption of whole PW and RW convenience bars for 8 weeks by overweight and obese adults with chronic inflammation induced modest and more pronounced reductions in plasma TNF-α in the pooled and RW participants, respectively. Plasma IL-6 and fasting glucose concentrations were reduced significantly only in the PW group. Both groups had comparable levels of plasma phenolic metabolites and oxidative stress markers at baseline and these remained unchanged with 4 and 8 weeks of RW product consumption. When data from both groups were pooled, improved plasma TNF-α, GSH and adiponectin were observed, pointing to the benefits of a high-fibre, whole-grain intervention. In general, these results support that 8-week consumption of whole-grain wheat products confers positive health impacts in terms of inflammation and oxidative stress, with modestly greater effects for the PW variety.

British Journal of Nutrition

References

1. Seal CJ & Brownlee IA (2015) Whole-grain foods and chronic disease: evidence from epidemiological and intervention studies. *Proc Nutr Soc* **74**, 313–319.
2. Jonnalagadda SS, Harnack L, Hai Liu R, *et al.* (2011) Putting the whole grain puzzle together: health benefits associated with whole grains – summary of American Society for Nutrition 2010 Satellite Symposium. *J Nutr* **141**, 1011S–1022S.
3. Threapleton DE, Greenwood DC, Evans CEL, *et al.* (2013) Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ* **347**, f6879.
4. Călinoiu LF & Vodnar DC (2018) Whole grains and phenolic acids: a review on bioactivity, functionality, health benefits and bioavailability. *Nutrients* **10**, 1615.
5. Dykes L & Rooney LW (2007) Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World* **52**, 105.
6. Masisi K, Beta T & Moghadasian MH (2016) Antioxidant properties of diverse cereal grains: a review on *in vitro* and *in vivo* studies. *Food Chem* **196**, 90–97.
7. Lee YM, Han SI, Song BC, *et al.* (2015) Bioactives in commonly consumed cereal grains: implications for oxidative stress and inflammation. *J Med Food* **18**, 1179–1186.
8. Hoevenaars FPM, Esser D, Schutte S, *et al.* (2019) Whole grain wheat consumption affects postprandial inflammatory response in a randomized controlled trial in overweight and obese adults with mild hypercholesterolemia in the Graandioos Study. *J Nutr* **149**, 2133–2144.
9. Vitaglione P, Mennella I, Ferracane R, *et al.* (2015) Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. *Am J Clin Nutr* **101**, 251–261.
10. Vanegas SM, Meydani M, Barnett JB, *et al.* (2017) Substituting whole grains for refined grains in a 6-wk randomized trial has a modest effect on gut microbiota and immune and inflammatory markers of healthy adults. *Am J Clin Nutr* **105**, 635–650.
11. Kopf JC, Suhr MJ, Clarke J, *et al.* (2018) Role of whole grains versus fruits and vegetables in reducing subclinical inflammation and promoting gastrointestinal health in individuals affected by overweight and obesity: a randomized controlled trial. *Nutr J* **17**, 72.
12. Price RK, Wallace JMW, Hamill LL, *et al.* (2012) Evaluation of the effect of wheat aleurone-rich foods on markers of antioxidant status, inflammation and endothelial function in apparently healthy men and women. *Br J Nutr* **108**, 1644–1651.
13. Rice-Evans CA, Miller NJ & Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* **20**, 933–956.
14. Liu Q, Qiu Y & Beta T (2010) Comparison of antioxidant activities of different colored wheat grains and analysis of phenolic compounds. *J Agric Food Chem* **58**, 9235–9241.
15. Alan PA, Ofelia RS, Patricia T, *et al.* (2012) Cereal bran and wholegrain as a source of dietary fibre: technological and health aspects. *Int J Food Sci Nutr* **63**, 882–892.
16. Stevenson L, Phillips F, O'Sullivan K, *et al.* (2012) Wheat bran: its composition and benefits to health, a European perspective. *Int J Food Sci Nutr* **63**, 1001–1013.
17. Belobrajdic DP & Bird AR (2013) The potential role of phytochemicals in wholegrain cereals for the prevention of type-2 diabetes. *Nutr J* **12**, 62.
18. Samtiya M, Aluko RE & Dhewa T (2020) Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Prod Process Nutr* **2**, 1–14.
19. Soetan KO (2008) Pharmacological and other beneficial effects of anti-nutritional factors in plants – a review. *African J Biotechnol* **7**, 4713–4721.
20. Khoddami A, Truong HH, Liu SY, *et al.* (2015) Concentrations of specific phenolic compounds in six red sorghums influence nutrient utilisation in broiler chickens. *Anim Feed Sci Technol* **210**, 190–199.
21. Jacques PF, Cassidy A, Rogers G, *et al.* (2013) Higher dietary flavonol intake is associated with lower incidence of type 2 diabetes. *J Nutr* **143**, 1474–1480.
22. Abdel-Aal ESM, Young JC & Rabalski I (2006) Anthocyanin composition in black, blue, pink, purple, and red cereal grains. *J Agric Food Chem* **54**, 4696–4704.
23. Abdel-Aal ESM, Hucl P & Rabalski I (2018) Compositional and antioxidant properties of anthocyanin-rich products prepared from purple wheat. *Food Chem* **254**, 13–19.
24. Shipp J & Abdel-Aal E-SM (2010) Food applications and physiological effects of anthocyanins as functional food ingredients. *Open Food Sci J* **4**, 7–22.
25. Zhu F (2018) Anthocyanins in cereals: composition and health effects. *Food Res Int* **109**, 232–249.
26. Mrkvicova E, Pavlata L, Karásek F, *et al.* (2016) The influence of feeding purple wheat with higher content of anthocyanins on antioxidant status and selected enzyme activity of animals. *Acta Vet Brno* **85**, 371–376.
27. Wang Q, Han P, Zhang Phd M, *et al.* (2007) Supplementation of black rice pigment fraction improves antioxidant and anti-inflammatory status in patients with coronary heart disease. *Asia Pac J Clin Nutr* **16**, 295–301.
28. Liu Y, Qiu J, Yue Y, *et al.* (2018) Dietary black-grained wheat intake improves glycemic control and inflammatory profile in patients with type 2 diabetes: A randomized controlled trial. *Ther Clin Risk Manag* **14**, 247–256.
29. Gamel TH, Wright AJ, Pickard M, *et al.* (2019) Characterization of anthocyanin-containing purple wheat prototype products as functional foods with potential health benefits. *Cereal Chem* **97**, 34–38.
30. Gamel TH, Wright AJ, Tucker AJ, *et al.* (2019) Absorption and metabolites of anthocyanins and phenolic acids after consumption of purple wheat crackers and bars by healthy adults. *J Cereal Sci* **86**, 60–68.
31. Gomes JVP, Rigolon TCB, Souza MS da S, *et al.* (2019) Antiobesity effects of anthocyanins on mitochondrial biogenesis, inflammation, and oxidative stress: a systematic review. *Nutrition* **66**, 192–202.
32. Guo H & Ling W (2015) The update of anthocyanins on obesity and type 2 diabetes: experimental evidence and clinical perspectives. *Rev Endocr Metab Disord* **16**, 1–13.
33. AACC International (2010) *AACC Approved Methods of Analysis*, 11th ed. St Paul, MN: AACC International.
34. Abdel-Aal ESM & Rabalski I (2013) Effect of baking on free and bound phenolic acids in wholegrain bakery products. *J Cereal Sci* **57**, 312–318.
35. Ridker PM (2003) Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* **107**, 363–369.
36. Lefevre M & Jonnalagadda S (2012) Effect of whole grains on markers of subclinical inflammation. *Nutr Rev* **70**, 387–396.
37. Zhu Y, Ling W, Guo H, *et al.* (2013) Anti-inflammatory effect of purified dietary anthocyanin in adults with hypercholesterolemia: a randomized controlled trial. *Nutr Metab Cardiovasc Dis* **23**, 843–849.
38. Health Canada (2007) *Eating Well with Canada's Food Guide*. Ottawa: Health Canada.
39. Levy JC, Matthews DR & Hermans MP (1998) Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* **21**, 2191.
40. Leys C, Ley C, Klein O, *et al.* (2013) Detecting outliers: do not use standard deviation around the mean, use absolute deviation around the median. *J Exp Soc Psychol* **49**, 764–766.

41. Lambert D, Peterson B & Terpenning I (1991) Nondetects, detection limits, and the probability of detection. *J Am Stat Assoc* **86**, 266–277.

42. Zanoletti M, Abbasi Parizad P, Lavelli V, *et al.* (2017) Debranning of purple wheat: recovery of anthocyanin-rich fractions and their use in pasta production. *LWT - Food Sci Technol* **75**, 663–669.

43. Kadiri O (2017) A review on the status of the phenolic compounds and antioxidant capacity of the flour: effects of cereal processing. *Int J Food Prop* **20**, S798–S809.

44. Laddomada B, Caretto S & Mita G (2015) Wheat bran phenolic acids: bioavailability and stability in whole wheat-based foods. *Molecules* **20**, 15666–15685.

45. Stalmach A, Mullen W, Barron D, *et al.* (2009) Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans: identification of biomarkers of coffee consumption. *Drug Metab Dispos* **37**, 1749–1758.

46. Tian L, Tan Y, Chen G, *et al.* (2019) Metabolism of anthocyanins and consequent effects on the gut microbiota. *Crit Rev Food Sci Nutr* **59**, 982–991.

47. Kirwan JP, Malin SK, Scelsi AR, *et al.* (2016) A whole-grain diet reduces cardiovascular risk factors in overweight and obese adults: a randomized controlled trial. *J Nutr* **146**, 2244–2251.

48. Shah K & Shah P (2018) Effect of anthocyanin supplementations on lipid profile and inflammatory markers: a systematic review and meta-analysis of randomized controlled trials. *Cholesterol* **2018**, 8450793.

49. Jensen MK, Koh-Banerjee P, Franz M, *et al.* (2006) Whole grains, bran, and germ in relation to homocysteine and markers of glycemic control, lipids, and inflammation 1–3. *Am J Clin Nutr* **83**, 275–83.

50. Andersson A, Tengblad S, Karlströ B, *et al.* (2007) Whole-grain foods do not affect insulin sensitivity or markers of lipid peroxidation and inflammation in healthy, moderately overweight subjects. *J Nutr* **137**, 1401–1407.

51. Gaskins AJ, Mumford SL, Rovner AJ, *et al.* (2010) Whole grains are associated with serum concentrations of high sensitivity C-reactive protein among premenopausal women. *J Nutr* **140**, 1669–1676.

52. Sytar O, Bosko P, Živčák M, *et al.* (2018) Bioactive phytochemicals and antioxidant properties of the grains and sprouts of colored wheat genotypes. *Molecules* **23**, 2282.

53. Hakuna L, Doughan B, Escobedo JO, *et al.* (2015) A simple assay for glutathione in whole blood. *Analyst* **140**, 3339–3342.

54. Shimizu H, Kiyohara Y, Kato I, *et al.* (2004) Relationship between plasma glutathione levels and cardiovascular disease in a defined population: the Hisayama study. *Stroke* **35**, 2072–2077.

55. Yu L, Zhou K & Parry JW (2005) Inhibitory effects of wheat bran extracts on human LDL oxidation and free radicals. *LWT - Food Sci Technol* **38**, 463–470.

56. Liyana-Pathirana CM & Shahidi F (2007) The antioxidant potential of milling fractions from breadwheat and durum. *J Cereal Sci* **45**, 238–247.

57. Wang Y (2009) Prebiotics: present and future in food science and technology. *Food Res Int* **42**, 8–12.

58. Boue SM, Daigle KW, Chen MH, *et al.* (2016) Antidiabetic potential of purple and red rice (*Oryza sativa* L.) bran extracts. *J Agric Food Chem* **64**, 5345–5353.