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The effect of malting on phenolic compounds and radical scavenging activity in grains and breakfast cereals

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Abstract: Breakfast cereals are popular grain foods and sources of polyphenols. Malting alters polyphenol content and activity; however, effects are varied. The total polyphenol content (TPC), radical scavenging activity (RSA), and polyphenol profile were analyzed in unmalted and malted grains (wheat, barley, and sorghum) and breakfast cereals (wheat, barley) by Folin Ciocalteu Reagent (FCR), % inhibition of the free radical 2,2-diphenyl-1-picryl-hydrazyl, and high performance liquid chromatography. Higher TPC was observed in all malted grains and breakfast cereals compared with unmalted samples ($p < 0.05$). Higher RSA was also observed in all malted samples compared to unmalted samples ($p < 0.05$) except for wheat grain to malted wheat grain. In this study, malting induced additional polyphenols and antioxidant activity in grains and cereal products. Malted grain breakfast cereals may be practical sources of polyphenol antioxidants.

KEYWORDS
breakfast cereal, functional food, germination, grain, malted, polyphenols, ready-to-eat (RTE)

Practical Application: This study utilized malting in a unique way to investigate potential health benefits of polyphenols and antioxidant activity in grains (wheat, barley, and sorghum) and ready-to-eat breakfast cereals (wheat and barley). This study found that grains and breakfast cereals are important sources of antioxidant polyphenols, and these were significantly increased in malted varieties. Understanding this is important as grains and breakfast cereals are widely consumed staple foods. Consuming healthier grain products may be a practical strategy in reducing the risk of noncommunicable diseases such as colorectal cancer and type-2 diabetes, where wholegrain consumption may be important in prevention.

1 | INTRODUCTION

Wholegrain consumption is associated with reduced risk of chronic diseases such as cardiovascular disease, coronary heart disease (Aune et al., 2016; Schwingshackl et al., 2018), and colorectal cancer (Aune et al., 2011; World Cancer Research Fund & American Institute for Cancer Research, 2017). Wheat, barley, and sorghum grains are global staple foods (Food and Agriculture Organization of the United Nations [FAO], 1995, 2001) and are widely consumed as ready-to-eat (RTE) breakfast cereals. RTE breakfast cereals are among the key sources of dietary wholegrains in children (Bellisle et al., 2014; Hassan et al., 2020) and adults (Bellisle et al., 2014; Galea et al., 2017; Thane et al., 2007) and associated with improved nutrient intake and diet quality (Fayet-Moore et al., 2019; Zhu et al., 2019). The nutritional composition of breakfast cereals has improved over time in some studies (Croisier et al., 2021) but also shown varied (E. Wang et al., 2015) or no improvement (Chepulis et al., 2017) in others. This highlights the potential of RTE breakfast cereals to contribute to public health nutritional outcomes via industry partnerships in supporting national healthy eating guidelines (Australian Government Department of Health, 2021; Croisier et al., 2021).

The protective effects of wholegrains in reducing risk of chronic metabolic disease states may be attributed in part to the biological effects of polyphenols. Polyphenols are bioactive phenolic compounds formed from secondary metabolism in plants comprising an aromatic ring(s) with attached hydroxyl groups (Andres-Lacueva et al., 2010; Crozier et al., 2009; Manach et al., 2004). Polyphenols have demonstrated antioxidant activity by their ability to reduce reactive oxygen species (ROS) (Araujo et al., 2011; Weng & Yen, 2012) and are recognized for their potential role in disease prevention (Fardet, 2010; Scalbert et al., 2005) including inhibition of carcinogenesis (Fantini et al., 2015) and chemoprevention (Rao et al., 2018). In particular, polyphenols may play a role in targeting colorectal cancer (CRC), an association shared with wholegrains, by acting on colonic microbiota (Bhat & Kapila, 2017; Fernández-Navarro et al., 2018), inflammation (Little et al., 2015; Maleki et al., 2019; Owczarek & Lewandowska, 2017), and CRC precursor conditions (Bobe et al., 2008; Fang et al., 2019; Kaulmann & Bohn, 2016).

Grains and cereal foods are an important source of dietary polyphenols (Tetens et al., 2013; Zamora-Ros et al., 2016; Ziauddeen et al., 2018); however, the polyphenol content and composition varies between grain type (Shewry et al., 2013), and can also be altered through processing methods such as germination and malting (Donkor et al., 2012; Kok et al., 2019; Nelson et al., 2016).

Wheat, barley, and sorghum grains are used as raw materials for germination and malting to improve nutritional content and flavor (Wannenmacher et al., 2018) and are widely eaten as staple foods (FAO, 1995, 2001). Malted grains are a type of germinated (sprouted) grain that have undergone a controlled process of steeping, germination, and kilning (FAO, 2009) and germinated and malted grains are considered wholegrain if meeting specific criteria (Cereals & Grains Association, 2022). Malting protocols vary according to commercial specifications and maltsters' application, and as an example may include steeping for 24 h (FAO, 2009; Yousif & Evans, 2020), germination for 88 h (Yousif & Evans, 2020) to 96 h (FAO, 2009), and kilning for 24 h (FAO, 2009; Yousif & Evans, 2020) at up to 80°C (Yousif & Evans, 2020). Malting variations and grain type will impact biochemical outcomes (Lu et al., 2007; Tomkova-Drabkova et al., 2016), but generally results in degradation of starch to sugars, protein alterations, and synthesis of enzymes (FAO, 2009; Yang et al., 2021). Germination may also improve the nutritional profile of grains (FAO, 2001; Hotz & Gibson, 2007) and polyphenol content and activity (Donkor et al., 2012).

Germinated grains contain numerous polyphenols as bound and soluble compounds, including hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids (Gan et al., 2019; Lu et al., 2007; Tomkova-Drabkova et al., 2016). Polyphenol profiles vary in germinated grains due to germination conditions such as time and temperature (Cevallos-Casals & Cisneros-Zevallos, 2010; Donkor et al., 2012; Tomkova-Drabkova et al., 2016), degree of polyphenol reactivity (Tomkova-Drabkova et al., 2016; Wannenmacher et al., 2018), and grain variety (Donkor et al., 2012; Tomkova-Drabkova et al., 2016).

Breakfast cereals are key contributors to wholegrain and nutritional intakes (Fayet-Moore et al., 2019; Galea et al., 2017); however, little is known about their polyphenol content. Understanding how germination and malting impacts grain polyphenols in consumer products may provide insights into modulating bioactive compounds for dietary prevention of chronic diseases. The aim of this study was to measure the differences in total polyphenol content (TPC), in vitro radical scavenging activity (RSA), and quantify the polyphenol profile of wheat, barley, and sorghum extracts in raw and malted forms, as well as RTE breakfast cereals made from wheat and barley raw and malted grains. It was hypothesized that malting would significantly increase the TPC and RSA of grains and cereal foods.

2 | MATERIALS AND METHODS

2.1 | Grains and products

Wheat (Lion), barley (Finniss), and white sorghum were analyzed in unmalted (control) and malted forms. Additionally, RTE breakfast cereals made from control wheat, malted wheat, and control barley and malted barley were also analyzed. The malted grains were prepared by controlled steeping, germination (20°C for 96 h), and kilning (55°C to 77°C), at an Australian malting facility in line with other standard commercial protocols (FAO, 2009; Yousif & Evans, 2020). RTE breakfast cereals were manufactured by Sanitarium Health and Wellbeing Company™ (Berkeley Vale, Australia) according to commercial protocol, where whole grains were steamed at 140°C for 60 min, rolled into flakes, and pressed into rectangular biscuits which were then dried in a toasting oven to 5% moisture. Malted wheat RTE test cereals had been manufactured previously, and malted barley RTE test cereals were selected as a feasible comparator.

2.2 | Proximate and nutrient analysis

Proximate and nutrient analysis of wheat and barley grains and their breakfast cereals was performed by food analysis laboratories accredited by the Australian National Association of Testing Authorities (NATA) and conducted in duplicate for all analyses except for total dietary fiber and insoluble fiber conducted in singlicate. Methods of the Association of Official Analytical Collaboration (AOAC) International (AOAC INTERNATIONAL) were used to analyze total dietary fiber (AOAC 991.43) (Lee et al., 1992), soluble fiber (external, AOAC 991.43) (Lee et al., 1992), and soluble component soluble fiber (AOAC 2017.16) (McCleary & Collaborators, 2019). Fat was estimated by automated Soxhlet using FOSS Soxtec; ash (550°C); minerals (Ca, Fe, Mg, K, Na, etc.) previously done by atomic absorption spectroscopy (AAS); protein by Kjeldahl method (nitrogen times 6.25); and moisture by air oven moisture (130°C). The results for these studies were calculated from raw values provided.

2.3 | Chemicals

Folin Ciocalteu Reagent (FCR), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, caffeic acid, (+)- catechin hydrate, p-coumaric acid, ellagic acid, (−)- epicatechin, ferulic acid, gallic acid, protocatechuic acid, syringic acid, and vanillic acid were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Phosphoric acid and sodium carbonate were purchased from Merck (Darmstadt, Germany). Methanol (99.8%) was purchased from Chem Supply (Gillman, SA, Australia). Acetonitrile (Supergradient HPLC Grade) was purchased from Scharlau/Scharlab S.L. (Sentmenat, Spain). Distilled water (MilliQ plus) was purchased from Millipore (Australia).

2.4 | Polyphenol extraction

The protocol for extraction was adapted from previously published protocols with a direct extraction method using 80% methanol (Alvarez-Jubete et al., 2010; Donkor et al., 2012; Hung et al., 2011). From each sample, ~5 g in duplicate were weighed and stored at −20°C, then lyophilized (FD300 Dynavac Eng. Pty. Ltd, Melbourne, Australia) and final weights recorded (Mettler Toledo AB204-S ANB03, Switzerland). The freeze-dried samples were manually crushed using a mortar and pestle, further ground to a fine powder using an electric wand mixer (Bamix ® of Switzerland, Swiss Line Universal Wand Mixer ESGE Ltd, CH-9517, Mettlen/Switzerland) followed by further manual grinding. The powders were each sieved to achieve a uniform particle size using 500 μm stainless steel sieve (Laboratory Test Sieve BS410/1986 serial number 863985, 500-μm brass metal frame Pat. No. 667924 Endecotts Ltd, London, England), placed in labeled falcon tubes, and protected from light. After sieving, any remaining particles >500 μm were returned to mortar for further crushing and then sieving until most of the particles could be sieved. Remaining samples impervious to crushing (>500 μm) were added to each original powdered sample with weights recorded prior to addition. In between different samples, the grinding and sieving equipment was washed with warm, soapy water, and dried in a laboratory oven at 60°C for 30–45 min.

For the extraction, 1.0 g of powdered sample was ground in a mortar with 1 ml distilled water for 1 min, then 4 ml 100% methanol added and ground for 2 min. The contents were poured off into a labeled tube and the mortar was rinsed with 2.5 ml 80% methanol. The samples were vortexed for 5 min on level 8 then centrifuged 10 min at 2266 × g relative centrifugal force (RCF) at 18–20°C. The supernatant was poured off into a labeled tube, and the remaining pellet underwent secondary extraction. The supernatants were pooled and filtered using 0.45 μm syringe filters (Schleicher & Schuell GmbH, Dassel, Germany). The extracts were made up to 10 ml with 80% methanol, labeled, sealed with parafilm, and wrapped in aluminum foil to protect from light. Samples were immediately stored at −18°C for further analysis. New extracts were made prior to the final Folin and DPPH experiments.

2.5 | Total polyphenol content

The total polyphenol determination method using FCR was adapted from previously published protocols (Angioloni & Collar, 2011; Donkor et al., 2012) with minor modifications. Briefly, 0.5 ml test sample and 0.5 ml FCR were combined, swirled for 3 min to mix, followed by the addition of 10 ml Na_2CO_3 solution and 5 ml distilled water. Duplicate negative control samples were prepared comprising all reagents, minus the test samples. The mixture was thoroughly shaken and incubated for 60 min at room temperature before centrifuging at 2266 × g RCF at 20°C for 7 min. The supernatant was transferred to 1 ml cuvettes (Kartell, 1941, Italy) and absorbance measured at 750 nm (Spectrophotometer BiochromLibraS12, Biochrom Ltd, Cambridge, England). A standard curve was prepared from serially diluted gallic acid (4.6–300 μg/ml) (Singleton & Rossi, 1965). The results were combined for analysis from three experiments using five independent samples and negative controls ($n = 2$).

Total polyphenol content was expressed as gallic acid equivalents (GAE) (Angioloni & Collar, 2011) based on the linear relationship between absorbance and standard curve concentration. The straight-line regression equation was used against sample absorbance to determine the sample concentration in standard equivalents. Absorbance was linearly correlated with concentration.

$$y = 0.2946\,(m)\,x + 0.0647\,(p)\,, R^2 = 0.9994$$

(y = Absorbance, m = gradient slope, x = unknown concentration, p = constant).

2.6 | Radical scavenging activity

Determination of radical scavenging activity was measured by DPPH assay, adapted from previously published methods (Donkor et al., 2012; Hung et al., 2011) with minor modifications. Controls comprised reagents (DPPH and methanol) without test samples. All samples including controls and standards were measured in duplicate with averages taken of the duplicate measures.

A standard curve was prepared from serially diluted gallic acid (4.6–300 μg/ml); 0.075 mM DPPH/methanol solution was prepared and stored in a flask covered with aluminum foil in the dark at 4°C between measurements. The blank/negative control was prepared (3.9 ml DPPH and 0.1 ml methanol) and absorbance recorded at Time-0 (UV/Vis spectrophotometer, Biochrom, England) and then incubated for 30 min. The samples were incorporated with DPPH, 0.1 ml phenolic extract, 3.9 ml DPPH solution, and the resulting mixture was shaken vigorously. The samples were then incubated in the dark covered with aluminum foil at ambient temperature for 30 min, when absorbance was measured at 515 nm. The % inhibition was calculated as follows:

$$\%\text{DPPH scavenging}\,(I) = \{(Abs\,(t\ =\ 0) - Abs\,(t = 30)\ /\ Abs\,(t = 0)\ \times 100$$

where I: intensity of the light passing through the sample; $Abs\,(t = 0)$ = absorbance of DPPH + methanol at $t = 0$ min; Abs (t = 30) = absorbance of DPPH + phenolic extracts at $t = 30$ min.

The inhibition was expressed as GAE and a standard curve constructed. The results from three repeated experiments were pooled for analysis.

2.7 | Polyphenol profile

Quantification of polyphenol profile by reverse phase high performance liquid chromatography (RP-HPLC) was adapted from previously published protocols (Amici et al., 2008; Donkor et al., 2012) with minor modifications of additional wash steps between sampling. Standards of polyphenols were prepared to 1000 μg/ml in methanol and serially diluted to 125, 250, 500, and 750 μg/ml, and stored at −18°C. Methanolic samples and standards were filtered through 0.2 μm membrane syringe filters (Schleicher & Schuell GmbH, Dassel, Germany). A Varian HPLC system comprising of a Varian 9100 sampler and a 9012 solvent delivery system (Varian Analytical Instruments, California, Walnut Creek, USA) was used to analyze the standards and sample solutions separately. Ten microliters volume of each sample were injected onto a C18 Phenomonex Luna column, 250×4 mm, 2.5 μm porosity, fitted with an Ultra-Sep ES RP18 guard column (Phenomenex Australia Pty Ltd, Lane Cove, NSW, Australia). Mobile phases comprised of (A) 0.3% phosphoric acid and (B) acetonitrile (CH_3CN) were each vacuum filtered through 0.2-μm membrane filters. The solutions were dispensed in three stages with gradient elution as follows: from 10% to 20% of B in 45 min, 20% to 60% of B in 20 min then 60% to 90% of B in 20 min. The flow rate was 0.7 ml/min at room temperature and samples measured in duplicate, with three washes in between sampling using mobile phase A. Detection was via a diode array detector (Varian Associates, Sunnyvale, California, USA), allowing a range of wavelengths to be scanned with a maximum detection at 220 nm. Each sample was compared with reference standard retention times of caffeic acid (CA), catechin (C), ellagic acid (EA), epicatechin (EC), ferulic acid (FA), gallic acid (GA), luteolin (L),

protocatechuic acid (PA), p-coumaric acid (PC), syringic acid (SA), and vanillic acid (VA), that were serially diluted between 125 μg/ml and 1000 μg/ml, to create calibration curves. The results from two repeated experiments of five independent samples were pooled for analysis.

2.8 | Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics v. 25 (IBM). To determine whether malting made a significant difference to TPC or RSA, an independent sample t-test was performed. To determine whether there was a difference between control samples and malted samples, a one-way ANOVA was performed with Tukey's Honestly Significant Difference (HSD) post-hoc analysis. Significance was set at $p < 0.05$. Data was expressed as mean ± standard error of the mean (SEM).

3 | RESULTS AND DISCUSSION

3.1 | Proximate and nutrient analysis

Proximate and nutrient analysis of wheat and barley samples are summarized in Table 1. Malting was associated with minimal effects on nutrients except for dietary fiber, where control samples of barley grain and barley breakfast cereals contained greater amounts of total dietary fiber and soluble dietary fiber. Barley control grain and breakfast cereal had the highest mean values for total dietary fiber (18% and 14%, respectively) and soluble dietary fiber (7.9 g/100 g and 5.4 g/100 g, respectively) and in agreement with other barley studies for total dietary fiber (~15–24%) and soluble dietary fiber β-glucan levels (~4.0–6.5%) in barley (Andersson et al., 2008). Dietary fiber is associated with favorable physiological effects including bowel laxation, blood glucose modulation, and blood cholesterol reduction (FSANZ, 2014; Ho et al., 2016; National Health and Medical Research Council, 2006). The higher soluble dietary fiber in the control barley breakfast cereal (7.9 g/100 g) compared with the malted barley cereal (3.6 g/100 g) may be partly attributed to solubilization that is suggested to take place during longer germination periods in malting of barley, associated with breakdown of β-glucan (Hübner et al., 2010). Proximate and nutrient analysis of wheat and barley and in grains, in general, play a crucial role in assessing their nutritional significance. As various grains are used as food along with their health benefits, evaluating the nutritional significance can help to understand the value of these grains (Donkor et al., 2012).

TABLE 1 Proximate and nutrient analysis of wheat and barley samples

	Wheat grain		Wheat breakfast		Barley grain		Barley breakfast	
	Control	Malted	Control	Malted	Control	Malted	Control	Malted
Energy (kJ/100 g)	1433.00 ± 1.00	1537.00 ± 1.00	1529.50 ± 0.50	1545.50 ± 0.50	1376.50 ± 0.50	1524.00 ± 0.00	1541.50 ± 1.50	1549.50 ± 0.50
Protein (g/100 g)	11.75 ± 0.05	12.05 ± 0.25	12.45 ± 0.05	12.15 ± 0.05	12.25 ± 0.05	12.75 ± 0.05	13.00 ± 0.00	12.60 ± 0.00
Fat (g/100 g)	1.30 ± 0.00	1.25 ± 0.05	1.65 ± 0.05	1.50 ± 0.00	1.30 ± 0.00	1.35 ± 0.05	2.10 ± 0.10	2.20 ± 0.10
Carbohydrate (g/100 g)	63.30 ± 0.00	69.90 ± 0.20	67.90 ± 0.10	69.70 ± 0.00	56.80 ± 0.00	67.35 ± 0.15	65.85 ± 0.15	68.20 ± 0.20
Total dietary fiber (%)	12.20	10.80	11.40	10.90	18.00	12.60	14.00	10.50
Soluble fiber (g/100 g)	2.20	2.60	3.60	3.40	5.40	2.90	7.90	3.60
Insoluble fiber (g/100 g)	10.00	8.20	7.80	7.50	12.60	9.70	6.10	6.90
Ash (g/100 g)	1.28 ± 0.05	1.16 ± 0.02	1.30 ± 0.01	1.16 ± 0.02	1.67 ± 0.01	1.57 ± 0.02	1.54 ± 0.02	1.42 ± 0.01
Calcium (mg/100 g)	29.45 ± 0.05	32.7 ± 0.20	25.00 ± 0.10	28.85 ± 0.45	28.10 ± 0.10	28.10 ± 0.50	28.30 ± 0.40	27.75 ± 0.35
Iron (mg/100 g)	3.90 ± 0.20	2.80 ± 0.00	4.05 ± 0.05	3.05 ± 0.05	4.00 ± 0.50	4.10 ± 0.10	4.45 ± 0.25	4.35 ± 0.05
Magnesium (mg/100 g)	92.95 ± 2.25	113.10 ± 4.10	109.35 ± 2.65	110.60 ± 4.60	109.80 ± 0.20	119.95 ± 0.05	122.00 ± 2.00	119.85 ± 1.15
Potassium (mg/100 g)	339.50 ± 2.50	302.00 ± 3.00	359.00 ± 1.00	305.00 ± 5.00	380.50 ± 6.50	343.50 ± 5.50	414.00 ± 5.00	331.00 ± 2.00
Sodium (mg/100 g)	2.60 ± 0.10	3.40 ± 0.30	4.70 ± 0.70	3.70 ± 0.00	14.15 ± 1.15	8.25 ± 0.05	18.15 ± 0.35	7.40 ± 0.50
Moisture (%)	10.13 ± 0.01	4.85 ± 0.03	5.30 ± 0.04	4.56 ± 0.05	10.00 ± 0.04	4.34 ± 0.04	3.51 ± 0.02	5.11 ± 0.09

Note: Proximate and nutrient analysis calculated as mean ± SEM, $n = 2$ for all groups except fiber $n = 1$. Carbohydrate excludes fiber.

TABLE 2 Total polyphenol content (TPC)[a] of control and malted wheat, barley, and sorghum grain samples

Sample	TPC GAE (μg/ml)	
	Control	Malted
Wheat grain	0.36 ± 0.04^{Ca}	1.01 ± 0.04^{Db}
Wheat breakfast	0.72 ± 0.02^{Ba}	2.02 ± 0.08^{Bb}
Barley grain	0.86 ± 0.03^{Ba}	1.45 ± 0.05^{Cb}
Barley breakfast	1.38 ± 0.06^{Aa}	2.38 ± 0.04^{Ab}
Sorghum grain	0.44 ± 0.03^{Ca}	0.89 ± 0.05^{Db}

Note: Data is presented as mean ± SEM, $n = 5$ for all groups. Different capital letters within columns indicate significant difference ($p < 0.05$); different small letters within rows indicate significant difference ($p < 0.05$).
[a]Total polyphenol content measured via gallic acid equivalents (GAE).

TABLE 3 % Inhibition against 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical[a]

Sample	% DPPH inhibition (GAE)	
	Control	Malted
Wheat grain	2.23 ± 1.91^{Ba}	3.08 ± 3.08^{Ca}
Wheat breakfast	14.77 ± 1.49^{Aa}	30.61 ± 1.53^{Ab}
Barley grain	19.23 ± 3.12^{Aa}	27.37 ± 2.52^{Ab}
Barley breakfast	10.54 ± 6.16^{Aa}	27.19 ± 4.37^{Ab}
Sorghum grain	15.68 ± 1.03^{Aa}	18.23 ± 0.92^{Bb}

Note: Data is presented as mean ± SEM, n = 5 for all groups. Different capital letters within columns indicate significant difference ($p < 0.05$); different small letters within rows indicate significant difference ($p < 0.05$).
[a]% DPPH inhibition measured via gallic acid equivalents (GAE).

3.2 | Total polyphenol content

In this study, we investigated the effect of malting on total polyphenol content (TPC) in wheat, barley, and sorghum grains and breakfast cereals made from wheat and barley. The effect of malting was associated with a significant increase in TPC in all malted samples compared with controls (Table 2). Malted barley breakfast cereal showed the highest mean TPC 2.38 ± 0.04 SEM μg/ml, and this difference was significant compared with control ($p < 0.05$). A significant difference was also observed between the control grain group means ($p < 0.05$). Post-hoc analysis suggested there was a significant difference in TPC between all controls and their malted samples ($p < 0.05$) as well as grains and their breakfast cereals ($p < 0.05$). The increase in TPC in germinated and malted grains compared with control grains is generally consistent with other studies in grains (Donkor et al., 2012) and breakfast cereals (Nelson et al., 2016) and may be attributed to the increased enzyme activity during germination and Maillard reaction during kilning that produces endogenous antioxidants (Wannenmacher et al., 2018; Zhao et al., 2006).

3.3 | Radical scavenging activity

Polyphenols are recognized for their ability to act as chain-breaking antioxidants by phenolic hydroxyl groups acting as electron donors (Chen et al., 2020) and may offer possible protection against diseases associated with oxidative damage (Azqueta & Collins, 2016; Dai & Mumper, 2010; Koziara et al., 2019). Free radical scavenging activity by the antioxidants in the samples was measured against the DPPH radical.

All samples in this study exerted inhibitory effects against the in vitro free radical activity of DPPH (Table 3). The mean % DPPH inhibition ± SEM (GAE) for control wheat breakfast cereal was 14.77% ± 1.49 and for malted wheat breakfast cereal was 30.61% ± 1.53, which was significantly higher in the malted cereal ($p < 0.05$). A significant difference was observed between the group means in control grains ($p < 0.05$). Post-hoc analysis indicated a significant difference in RSA between wheat and barley grain controls ($p < 0.05$), and between wheat and sorghum grain controls ($p < 0.05$), with both barley and sorghum control grain inhibition significantly higher than for wheat. There was also a significant difference between control wheat samples and their respective breakfast cereals and all control and malted samples ($p < 0.05$) except for between control wheat grain and malted wheat grain. However, antioxidant capacity has been found to vary with the effect of the reaction system, suggested to be related to the formation of intramolecular hydrogen bonds between functional groups (4-OH and o-methoxy groups) (Chen et al., 2020) and therefore may differ in other systems.

The relationship between polyphenol concentration and radical scavenging activity was observed to follow similar trends when plotted against each other (Figure 1), where greater TPC is reflected in greater % DPPH inhibition. These trends are consistent with other studies finding increased total polyphenols and antioxidant activity associated with germination and malting of grains (Cevallos-Casals & Cisneros-Zevallos, 2010; Hung et al., 2012; Karwasra et al.,2018; Panfil et al., 2014).

While increased TPC and RSA has been reported in this and other germination and malting studies, the health implications in humans of higher polyphenol concentrations in cereal foods are unclear. Wide variability has been reported in polyphenol bioavailability, plasma concentrations, urinary excretion, and elimination half-life (Kern et al., 2003; Manach et al., 2005). Bioavailability may be limited in humans as reported with limited hydroxycinnamate absorption following consumption of a high-bran wheat cereal that may reflect only the soluble and free components (Kern et al., 2003). Individual variation may be influenced by individual health status (Martini et al., 2019),

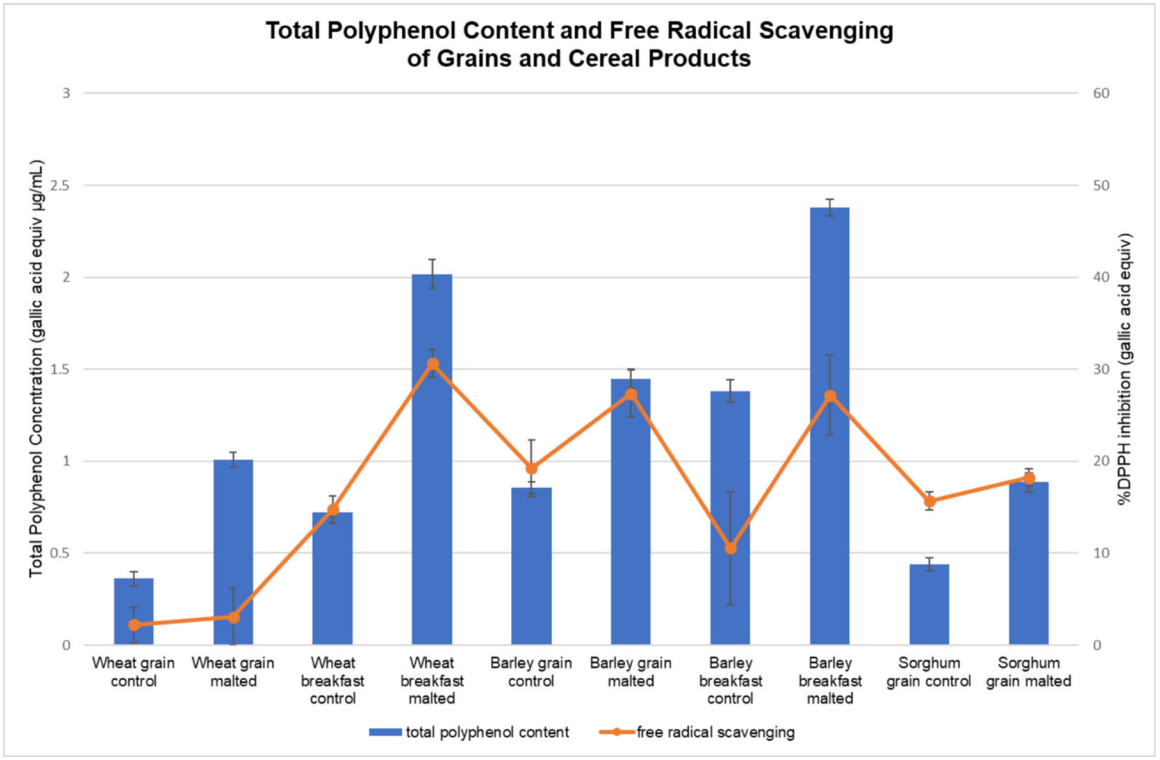

FIGURE 1 Total polyphenol content and free radical scavenging of grains and cereal products. Total polyphenol concentration expressed as gallic acid equivalents (µg/ml) and free radical scavenging expressed as % DPPH inhibition. Data presented as mean ± SEM

function of host gut microbiota, metabolizing enzymes, age, and other factors (Manach et al., 2017). While evidence from epidemiological studies have shown higher dietary polyphenol intakes are associated with reduced risk of several noncommunicable diseases including colorectal cancer, the findings are often inconsistent (Arts & Hollman, 2005; Grosso et al., 2016) and confounded by the complexity of the vast number of compounds in foods when investigating diet–health interactions (Arts & Hollman, 2005).

3.4 | Polyphenol profile

The polyphenol profiles of grains and malted grains extracts were compared using RP-HPLC against 11 reference standards, reflecting the potential functional importance of the different grains (Figure 2). Caffeic acid and syringic acid were co-eluting and clarified by spiking each in separate runs omitting or including luteolin. The elution pattern of standards was similar in other studies for the same compounds (Donkor et al., 2012; Hardin & Stutte, 1980).

Variations in polyphenol profiles were observed among the samples (Table 4). Several peaks of interest were identified based on retention times of the standards, as shown for barley grain (Figure 3). Luteolin and catechin were the most prevalent compounds detected, notably in barley grains followed by wheat. Luteolin may be associated with health benefits including improved outcomes in glycolipid metabolic disorders (Z. Wang et al., 2021). Much of the health benefits of catechins are associated with tea (Pedro et al., 2020) and while less is known about health effects of catechin from cereal foods, catechin appears to play a key role on barley flour dough discoloration (Quinde-Axtell & Baik, 2006).

Ferulic acid is recognized as the most abundant phenolic compound in raw and germinated grains (Donkor et al., 2012; Rico et al., 2020) and may have beneficial cardiovascular health effects in humans, including improved lipid parameters, decreased markers of oxidative stress, and inflammation (Bumrungpert et al., 2018) and enhancing vascular function (Turner et al., 2020). In the current study, detection of ferulic acid was comparatively limited across the samples (Table 4), consistent with decreases in ferulic acid reported during malting of barley following kilning (Tomkova-Drabkova et al., 2016) and in malted wheat compared with native wheat (Rao & Muralikrishna, 2004). Decreases in ferulic acid may be associated with induction of phenolic acid esterases during germination (Rao & Muralikrishna, 2004) and may vary depending on the kilning conditions (Inns et al., 2011). Lower concentrations of ferulic acid in this study may also have been underestimated due to extraction process without acid or

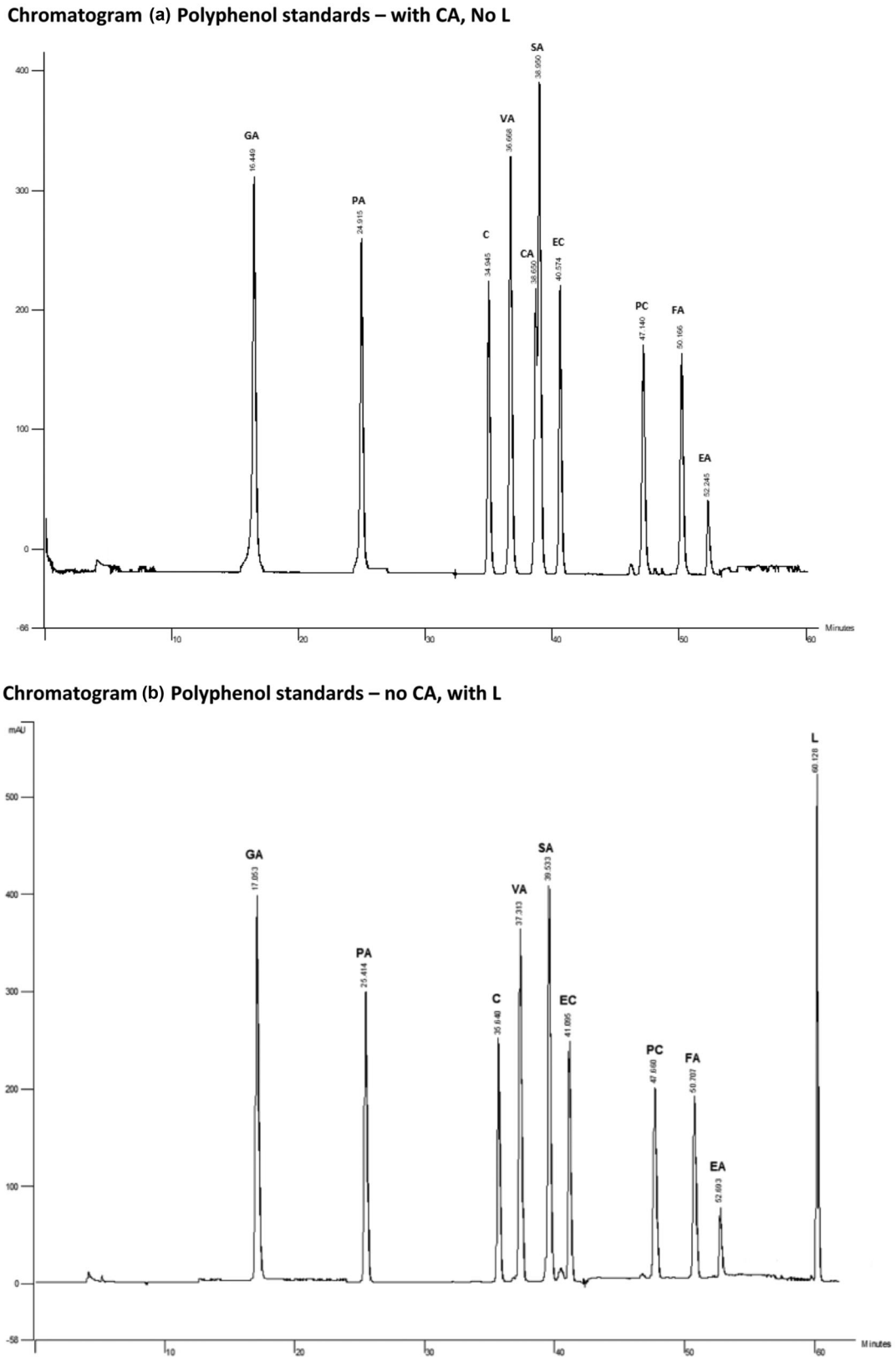

FIGURE 2 HPLC analysis – polyphenol standard chromatograms. Chromatogram A: elution profile of polyphenol standards: CA: caffeic acid; C: catechin; EA: ellagic acid; EC: epicatechin; FA: ferulic acid; GA: gallic acid; PC: p-coumaric acid; PA: protocatechuic acid; SA syringic acid; VA: vanillic acid. Chromatogram B: As for A, but including luteolin (L) and omitting caffeic acid (CA). Detection was achieved via a diode array detector (Varian Associates) at 220 nm

TABLE 4 Quantification of polyphenols in grains, malted grains, and breakfast cereals by reversed-phase high performance liquid chromatography (RP-HPLC)[a]

Mean RT (min)	**16.5**	**25.1**	**35.5**	**37.6**	**39.2**	**39.4**	**40.9**	**47.6**	**50.9**	**52.4**	**59.8**
	GA	PA	C	VA	CA	SA	EC	PC	FA	EA	L
	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)	(μg/ml)
Wheat											
WGC	ND	ND	ND	0.12 ± 0.12	ND	ND	ND	ND	0.07 ± 0.07	1.43 ± 1.13	0.60 ± 0.55
WGM	ND	ND	ND	0.33 ± 0.33	0.59 ± 0.59	ND	0.41 ± 0.41	ND	ND	ND	3.69 ± 3.41
WBC	ND	ND	ND	ND	0.09 ± 0.09	ND	ND	ND	ND	ND	0.42 ± 0.30
WBM	ND	ND	0.56 ± 0.36	ND	ND	ND	ND	ND	ND	0.31 ± 0.20	0.18 ± 0.11
Barley											
BGC	0.48 ± 0.48	0.39 ± 0.34	5.21 ± 0.81	0.07 ± 0.07	0.12 ± 0.12	ND	0.16 ± 0.16	0.15 ± 0.09	0.16 ± 0.10	ND	0.14 ± 0.08
BGM	ND	0.41 ± 0.41	3.08 ± 0.99	ND	ND	ND	ND	ND	ND	ND	0.05 ± 0.05
BBC [a]	2.06 ± 2.91	0.33 ± 0.33	3.26 ± 3.26	2.50 ± 1.31	ND	0.55 ± 0.55	0.28 ± 0.28	0.12 ± 0.12	0.21 ± 0.21	3.24 ± 3.24	0.22 ± 0.02
BBM [b]	ND	0.09 ± 0.09	2.99 ± 0.63	ND	ND	ND	0.34 ± 0.22	ND	ND	ND	ND
Sorghum											
SGC	ND	ND	0.09 ± 0.09	ND	0.22 ± 0.22	ND	ND	ND	ND	ND	0.29 ± 0.18
SGM	ND	ND	ND	1.4 ± 0.90	ND	ND	ND	ND	ND	ND	0.07 ± 0.04

Note: Data is presented as mean ± SEM, $n = 5$ for all groups except: [a] = n = 2; [b] = $n = 4$. No significant differences were detected for all groups compared with their control via independent samples t-test.

[a]Quantification of polyphenols by RP-HPLC measured via Gallic Acid Equivalents.

Abbreviations: BBC, barley breakfast control; BBM, barley breakfast malted; BGC, barley grain control; BGM, barley grain malted; C, catechin; EA, ellagic acid; CA, caffeic acid; EC, epicatechin; FA, ferulic acid; GA, gallic acid; ND, not detected; PA, protocatechuic acid; PC, p-coumaric acid; RT, retention time; SA, syringic acid; SGC, sorghum grain control; SGM, sorghum grain malted; VA, vanillic acid; WBC, wheat breakfast control; WBM, wheat breakfast malted; WGC, wheat grain control; WGM, wheat grain malted.

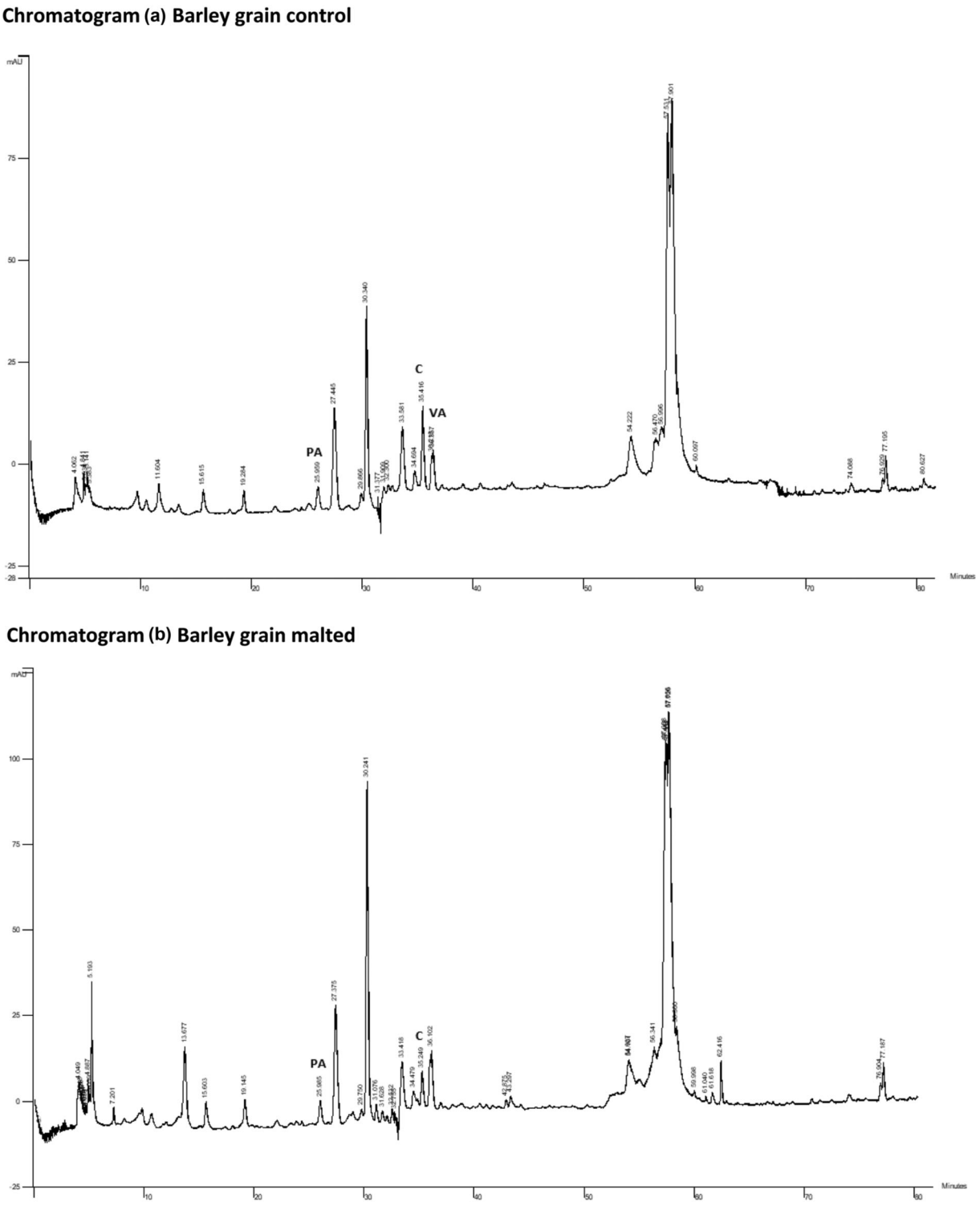

FIGURE 3 HPLC analysis – barley grain extracts chromatograms. Chromatogram A: elution profile of barley grain control hydroalcoholic extract, showing polyphenols identified against the standards: PA, C, and VA. Chromatogram B: elution profile of barley grain malted hydroalcoholic extract, showing polyphenols identified against the standards: PA and C. Detection was achieved via a diode array detector (Varian Associates) at 220 nm

alkali hydrolysis to liberate the bound compounds (Gan et al., 2019).

Variation in polyphenol profiles observed in this study are in agreement with previous studies that showed differences between raw grain varieties (Donkor et al., 2012; Li et al., 2008; Rao et al., 2018) and germinated/malted grains (Dicko et al., 2005; Donkor et al., 2012; Lu et al., 2007), although some differences with the values reported and directional change are apparent between studies. Differences in polyphenol concentration and profile may arise from genetic diversity and intervarietal differences in wheat (Shewry, 2009) and in sorghum (Dicko et al., 2005), differences in germination/malting conditions (Cevallos-Casals & Cisneros-Zevallos, 2010; Koehler et al., 2007; Lu et al., 2007), and individual polyphenol response to the treatment (Tomkova-Drabkova et al., 2016).

4 | CONCLUSION

Wholegrains and breakfast cereals contain diverse profiles of polyphenols that may be beneficial to health. The malting processes in this study were associated with increases in TPC in all samples and also yielded significant increases in RSA in most samples. The increased TPC in grains associated with malting may offer commercial potential for improving the content of bioactive polyphenols in popular breakfast foods for population health benefits, and may offer protection against diseases of oxidative stress such as CRC. Further experimental studies are warranted to assess the effects of food processing methods on polyphenols and antioxidant capacity, individual biological responses, and ultimately their ability to enhance health outcomes.

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AUTHOR CONTRIBUTIONS

Kristina Vingrys: conceptualization; data curation; formal analysis; investigation; methodology; project administration; visualization; writing – original draft; writing – review & editing. **Michael Mathai**: conceptualization; methodology; supervision; writing – review & editing. **John F. Ashton**: conceptualization; data curation; formal analysis; funding acquisition; methodology; resources; writing – review & editing. **Lily Stojanovska**: conceptualization; funding acquisition; methodology; supervision; writing – review & editing. **Todor Vasiljevic**: conceptualization; formal analysis; methodology; resources; supervision; visualization; writing – review & editing. **Andrew J. McAinch**: formal analysis; supervision; visualization; writing – review & editing. **Osaana N. Donkor**: conceptualization; formal analysis; methodology; resources; supervision; visualization; writing – review & editing.

CONFLICT OF INTEREST

Kristina Vingrys, Michael L. Mathai, Lily Stojanovska, Todor Vasiljevic, Andrew J. McAinch, and Osaana N. Donkor have no conflicts of interest. John F. Ashton is employed by Sanitarium Health and Wellbeing Company™ who supported this study by supplying the test cereals and contributed to the proximate and nutrition analyses.

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