Food Research International 197 (2024) 115149

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Micronization of wholewheat flour increases iron bioavailability from hydrothermally processed wheat flour dough

Mohamad F. Aslam [a], Sarah M. Arafsha [a,c], Sarah E. Berry [a], Balazs Bajka [a], Peter R. Ellis [b], Gladys O. Latunde-Dada [a,*], Paul A. Sharp [a,d,**]

[a] Department of Nutritional Sciences, King's College London, SE1 9NH, UK
[b] Biopolymers Group, Departments of Biochemistry and Nutritional Sciences, School of Life Course & Population Sciences, King's College London, SE1 9NH, UK
[c] Department of Clinical Nutrition, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[d] School of Food Science & Nutrition, University of Leeds, LS2 9JT, UK

ARTICLE INFO

Keywords:
Iron
Zinc
Bioaccessibility
Bioavailability
In vitro digestion
Caco-2 cells

ABSTRACT

Cereal products contribute significantly to dietary intake of essential minerals. In wheat, iron and zinc are stored in specific grain structures including the aleurone, scutellum and embryo. Wheat cell walls are resistant to digestion in the human gastrointestinal tract and therefore this study investigated the hypothesis that physical disruption of the cell walls would increase the bioaccessibility and bioavailability of iron and zinc from wheat-based foods. Flour was micronized using a combination of roller milling and a micro-mill and this reduced median particle size by two-thirds. Hydrothermally processed wheat flour doughs were subjected to *in vitro* digestion to determine mineral bioaccessibility. Mineral bioavailability from food digests was measured using human intestinal Caco-2 cells. Iron (but not zinc) bioavailability from wheat foods made using the micronized flour (2.5 ± 0.5 nmol/mg cell protein) was increased significantly compared with foods produced from standard milled flour (1.3 ± 0.1 nmol/mg cell protein; $P = 0.031$). Micronization of wheat flour has the potential to increase the absorption of the endogenous iron present in cereal foods and this might have health benefits for population groups with poor iron status.

1. Introduction

Cereals are important sources of energy in global diets and contribute significantly to mineral intakes. For example, up to 50 % of iron and 30 % of zinc in the UK diet is provided by cereal products, with the majority coming from wheat-based foods (e.g. bread, pasta) (Aslam, Ellis, Berry, Latunde-Dada, & Sharp, 2018). In wheat, iron is primarily localised to the aleurone layer and scutellum, while zinc is abundant in aleurone and the embryo of the mature wheat grain. However, only limited amounts of these minerals are present in the starchy endosperm (Balk et al., 2019).

Mineral bioaccessibility and bioavailability from wholegrain wheat flour are limited due to the presence of intact plant cell walls. In particular, the cell walls of the mineral-rich aleurone layer of wheat are relatively thick and robust and remain largely intact during milling and food processing (Brouns, Hemery, Price, & Anson, 2012). Several studies have shown that wheat cell walls, including those in the aleurone layer, are also resistant to digestion in the small intestine and therefore nutrients, including iron and zinc, that are physically encapsulated within wheat cells have low bioaccessibility (i.e. are not readily liberated during digestion) (Latunde-Dada et al., 2014; Edwards et al., 2015a,2021).

A series of elegant imaging studies have shown that iron and zinc in the aleurone, scutellum and embryo are strongly associated with phosphorus, most likely in the form of phytate (Moore et al., 2012; Neal et al., 2013; De Brier et al., 2016; Wan et al., 2022). Phytate (an anionic form of inositol hexakisphosphate) has been shown to be a potent inhibitor of iron and zinc bioavailability in both *in vitro* assays (Sreenivasulu, Raghu, Ravinder, & Nair, 2008; Christides & Sharp, 2013) and in human feeding studies (Hallberg, Brune, & Rossander, 1989; Brnić, Wegmüller, Zeder, Senti, & Hurrell, 2014). Thus, in wholegrain wheat products, the physical encapsulation of iron and zinc by cell walls together with the presence of high levels of phytate limits both bioaccessibility and bioavailability of minerals.

* Corresponding author.
** Corresponding author at: School of Food Science & Nutrition, University of Leeds, LS2 9JT, UK.
E-mail addresses: yemisi.latunde-dada@kcl.ac.uk (G.O. Latunde-Dada), P.A.Sharp@leeds.ac.uk (P.A. Sharp).

https://doi.org/10.1016/j.foodres.2024.115149
Received 18 June 2024; Received in revised form 23 September 2024; Accepted 25 September 2024
Available online 29 September 2024

Several strategies have been proposed to increase mineral content and bioavailability from cereal products including fortification, biofortification and genetic manipulation (Arafsha, Aslam, Ellis, Latunde-Dada, & Sharp, 2023). The purpose of this current study was to test the hypothesis that physical disruption of wheat cell walls through micro-milling of wheat grain would increase the bioavailability of the endogenous iron and zinc from hydrothermally processed wholewheat foods. Here we have used a well-characterised *in vitro* digestion/cell absorption model to investigate the effects of hydrothermal processing of wheat flour doughs on mineral bioaccessibility and bioavailability. Bioaccessibility of iron and zinc was measured as the amount of mineral liberated from the food matrix following *in vitro* digestion. Digestates were then added to human intestinal Caco-2 cells to measure iron and zinc uptake (bioavailability). Our studies showed that iron bioavailability from cooked foods was increased in products made using the micronized flour.

2. Methods & materials

2.1. Proximate analysis of wheat flour and preparation of food samples

All flours were prepared and supplied by Bühler AG (Switzerland). Swiss wholegrain wheat (Vollkornmehl Typ 1900) was used to produce wholewheat flours using either a standard roller milling process (STD) or using a Bühler Micromill (a modified roller mill containing 3 pairs of smooth rollers with one in each pair rotating faster than the other to increase the shearing forces) to yield micro-milled (MM) flour. Particle size analysis of the standard and micro-milled flours was carried out at Campden BRI (UK) using Dynamic Image Analysis (UKAS, based on ISO 13322-2:2006).

Proximate analysis of flours (technical duplicates) was performed by Campden BRI and included moisture (%, near-infrared spectroscopy; NIR, non UKAS, TES-CM-114 based on CCAT method 14), protein (%, NIR, non UKAS, TES-CM-114 based on CCAT method 14), dry gluten (%, gluten washing test, non UKAS, TES-CM-113 based on CCAT method 13), ash (total minerals, % dry matter basis, UKAS, TES-CM-112 based on CCAT method 12), damaged starch (Farrand (FU), UKAS, TES-CM-105 based on CCAT method 05), α–amylase (ceralpha (CU/g), UKAS, TES-CM-118 based on CCAT method 05) and Hagberg falling number (an indirect measurement of α-amylase activity, UKAS, TES-CM-106 based on CCAT method 06). The flours were kept refrigerated and removed from the fridge one hour before use to reach room temperature.

To make wheat doughs, 20 g of each flour was mixed with water (11.7 mL for standard flour; 12.2 mL for micro-milled flour) and doughs were kneaded by hand for 10 min and left to rest at 37 °C for 1 h. Samples of dough (approximately 5 g) were taken, rolled, and flattened into small discs (approximately 5 cm diameter, 0.5 cm thickness) and simmered in boiling tap water for 10 min, turning after 5 min. Samples were cooled and were then placed in aluminium dishes in an oven at 60 °C to remove moisture and achieve constant weights. Each cooked sample was ground in a coffee blender before use in experiments.

2.2. Iron and phytic acid content

Samples of dehydrated food powder (0.5 g) were weighed, topped up to 10 mL with a 1:1 mixture of nitric acid and de-ionised water and heated in a MARS 6 Microwave Reaction system (CEM Microwave Technology Ltd. UK) for 60 min. After cooling, 140 μL of the internal yttrium standard (100 ppm) was added and the solution was adjusted to a volume of 14 mL with distilled water. The iron and zinc contents (mg/100 g dry weight) of the samples were determined from a standard curve established using a multi-element standard solution (1 mg/mL; Thermo-Fisher) and inductively coupled plasma-optical emission spectrometer (ICP-OES, iCAP 6000, Thermo-Fisher).

Measurements of phytic acid and total phosphorus were performed using a commercially available test kit (K-PHYT) purchased from Megazyme (Ireland), and followed the method described by McKie and McCleary (2016). Briefly, 0.5 g of dehydrated food samples were solubilised overnight with HCl (0.66 M). Solubilised extracts were treated with phytase and alkaline phosphate to release available phosphate from phytic acid. Total phosphate was determined spectrophotometrically (absorbance, 655 nm) using ammonium molybdate and a calibration curve generated using standards of known phosphorus concentration. Values were corrected for free phosphorus levels in the sample and the calculation of phytic acid content (g/100 g dry weight) of the sample assumed that the remaining phosphorus was released exclusively from phytic acid and that phosphorus comprises 28.2 % of phytic acid.

2.3. In vitro digestion

Dehydrated food samples were subjected to simulated gastric and intestinal digestion as described previously (Latunde-Dada et al., 2014). For peptic digestion, 0.5 g of each sample was each added to 10 mL saline solution (140 mM NaCl and 5 mM KCl, pH 2.0), vortexed and left at room temperature for 15 min. The mixtures were then re-adjusted to pH 2.0 with HCl (1 M) and 0.5 mL pepsin (16 mg/mL in pH 2.0 saline) was added to initiate digestion. The samples were incubated in the dark at 37 °C for 90 min on a rocking platform. Subsequently, the pH was adjusted to 7.0 with $NaHCO_3$ (1 M) to inactivate the pepsin and 2.5 mL pancreatin-bile extract (1.4 mg/mL pancreatin and 8.5 mg/mL bile) was added. Each mixture was adjusted to 15 mL final volume using saline solution and incubated at 37 °C for a further 90 min.

2.4. Mineral bioaccessibility

Following *in vitro* digestion, samples were centrifuged at 1000 rpm for 10 min to remove undigested food and supernatant of each digestate was collected for further analysis. Total mineral release (i.e., bioaccessibility) was measured in digestates; 0.5 mL of each digestate was diluted in 1 mL HNO_3 (69 %). Sample volume was adjusted to 6 mL with HPLC ultrapure grade water (Sigma-Aldrich, UK), and the iron content of each sample was measured using the ICP-OES as described above.

2.5. Cell culture

Caco-2 cells (HTB-37) were acquired from the American Type Culture Collection (ATCC, Rockville, MD, USA) and were used for experiments between passages 25 and 40. Cells were maintained in Minimal Essential Medium (MEM) containing 10 % (v/v) heat-inactivated foetal bovine serum (FBS), 1 % (v/v) penicillin/streptomycin, 1 % (v/v) non-essential amino acids and 1 % (v/v) fungizone (all reagents were from Invitrogen, Paisley, UK). For experiments, cells were grown in 6-well plates and were used 14 days post-seeding.

2.6. Mineral bioavailability

Working solutions of each food digestate were prepared by diluting 1:1 with MEM and 2 mL of each working solution was added to individual wells on 6-well cell culture plates. Caco-2 cells were incubated with digests at 37 °C for 4 h. Following treatment, the Caco-2 cells were washed twice with PBS-EDTA to remove residual medium, and minerals adherent to the plates and the outside of the cells. The cells were lysed by adding 0.5 mL NaOH (50 mM) to each well and left to incubate at room temperature for 90 min. Afterwards, the cells were physically disrupted by drawing each sample through a pipette four times. A small sample of the treated cell lysate (25 μL) was collected for protein quantification. The remaining cell samples were transferred to screw-top tubes and were heated at 60 °C for 3 h in a sample concentrator; 200 μL HNO_3 (69 %) was added to each sample, vortexed for 3 s and digested on a heat block at 80 °C for 2 h. Afterwards, the samples were allowed to reach room temperature and were then mixed with 50 μL gallium (5 ppm; internal standard) and 4.75 mL Milli-Q water. The mineral contents in

the diluted cell samples were determined using ICP-MS by staff at the London Metallomics Facility (King's College London).

2.7. Fluorescence microscopy

Standard and micro-milled flours and prepared doughs were suspended in PBS containing 10 μg/mL calcofluor-white, fluorescein isothiocyanate (FITC), nile red and fast green FCF (Sigma-Aldrich Co, Poole, UK) to stain cell walls, starch, lipid and protein, respectively. Following incubation for 60 min at room temperature on a rotating mixer, samples were centrifuged and washed twice in PBS, mounted on slides and viewed using a Leica SP2 confocal microscope (Leica Microsystems, Mannheim, Germany). Samples were imaged using, λ_{ex} of 405 nm and λ_{em} of 410–480 nm for calcofluor, λ_{ex} of 488 nm and λ_{em} of 500–550 nm for FITC, λ_{ex} of 543 nm and λ_{em} of 550–630 nm for nile red, and λ_{ex} of 633 nm and λ_{em} of 640–750 nm for fast green. Image stacks of flour samples at 63× magnification and dough samples at 20x magnification were collected, and average z-projections were generated using Fiji image analysis software (https://fiji.sc/).

2.8. Statistics

Data were analysed with SigmaPlot (version 14.5). Comparison of means was conducted using Student's unpaired *t*-test or Two-way Analysis of Variance and Tukey's *post-hoc* test where appropriate. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Proximate analysis, mineral content, and physical characterisation of flours

Flour characterisation was carried out by Campden BRI (data are means of technical duplicates). There were no differences in the factors measured (protein, moisture, dry gluten, or ash, respectively) between the standard and micro-milled wholewheat flours. (Table 1). There was a small increase in damaged starch, and while Hagberg falling number was lower in the micro-milled flour, the values for both standard and micro-milled flours were above the acceptability threshold (250 s) for breadmaking. Water absorption capacity of the micro-milled flours was approximately 5 % higher than the standard milled flour due to the presence of smaller sized particles and greater exposure of water absorbing gluten proteins. Consequently a 5 % greater volume of water was used to produce the doughs from the micro-milled flour to ensure optimum mixing.

There were no statistical differences in the levels of iron in the standard and micro-milled flour samples, or following cooking, indicating that iron content was not affected by the milling or hydrothermal processes. In contrast, there was a small but significant decrease in zinc content following micro-milling in both the uncooked flour and hydrothermally processed doughs (Table 2).

Table 1
Characterisation of standard and micro-milled wholewheat flours.

	Standard wholewheat	Micro-milled wholewheat
Protein (%; rapid NIR analysis)	12.6	12.0
Moisture (%; rapid NIR analysis)	11.9	11.6
Damaged starch (Farrand units)	23	27
Dry gluten (%)	11.0	9.9
Ash (%; total mineral content)	1.52	1.58
Water absorption (%; Farinograph)	64.2	68.2
α-amylase (Ceralpha; CU/g)	0.17	0.15
Hagberg falling number (seconds)	390	362

Data are mean values of technical duplicates.

Samples of standard milled (median particle size: 378 μm) and micro-milled flour (median particle size: 126 μm) were used to produce wheat doughs (Fig. 1). The structure of the flours and doughs was visualised using fluorescence microscopy. Large amounts of intact cellular structure were observed in the standard milled flour and corresponding dough (Fig. 2A, C). In contrast, in the micro-milled flour and dough, cell structure was more fragmented (Fig. 2B, D).

3.2. Mineral bioaccessibility and bioavailability

Next, we assessed the effect of hydrothermal treatment (boiling) on iron and zinc bioaccessibility and bioavailability. In boiled samples, there was no significant difference in iron (Fig. 3A) or zinc (Fig. 3C) bioaccessibility following *in vitro* digestion. Iron bioavailability was significantly increased in samples made using micro-milled flour compared with standard milled flour (Fig. 3B). However, while zinc bioavailability was marginally higher in micro-milled samples, this did not reach statistical significance (Fig. 3D).

3.3. Phytic acid content of wheat flour and hydrothermally processed doughs

We hypothesised that micro-milling would influence phytic acid levels in the wholewheat flour and in the hydrothermally processed doughs. While total phytic acid levels were significantly reduced by hydrothermal processing, there was no significant differences between flour types in either the flour or the hydrothermally processed doughs (Table 2).

4. Discussion

The bioaccessibility and bioavailability of minerals from cereal products are limited by the presence of non-digestible cell walls encapsulating the intracellular minerals (Latunde-Dada et al., 2014) and the high levels of phytic acid in cereal-based foods, which forms insoluble complexes with minerals (reviewed in Balk et al., 2019). Wheat forms the basis for many foods and, according to the National Diet and Nutrition Survey in the UK (National Diet and Nutrition Survey, 2016), it is mostly consumed as bread and pasta and provides approximately 40 % of iron and 25 % of zinc in the diets of adults in the UK. Here we used standard and micronized flours to produce wheat doughs, which were then cooked in boiling water, to determine whether physical disruption of cereal plant cell walls could increase the bioaccessibility and bioavailability of iron and zinc from wheat-based foods.

Food structure and food processing, notably thermal and mechanical

Table 2
Iron, zinc and phytic acid content in wheat flour and hydrothermally processed doughs.

Hydrothermal treatment	Wheat flour type	Iron		Zinc		Phytic acid	
		mg/100 g				g/100 g	
		Mean	SD	Mean	SD	Mean	SD
Uncooked	STD	3.57	0.11	3.36	0.22	2.61	0.16
	MM	3.44	0.18	3.08[a]	0.08	2.52	0.03
Boiled	STD	3.87	0.34	2.95	0.19	1.31*	0.45
	MM	3.49	0.11	2.63[a]	0.05	1.18*	0.46

Data are means ± SD (n = 3–6) and were analysed using 2-way ANOVA and Tukey's *post-hoc* test. Micro-milling significantly decreased zinc, but not iron, content in both the uncooked flour and the hydrothermally processed doughs ([a]$P < 0.02$). Zinc levels were also lower in the hydrothermally processed doughs than the uncooked flours ($P < 0.01$). Hydrothermal processing decreased phytic acid content compared with levels in the corresponding raw flour (*$P < 0.01$). There was no significant effect of flour type on phytic acid levels.

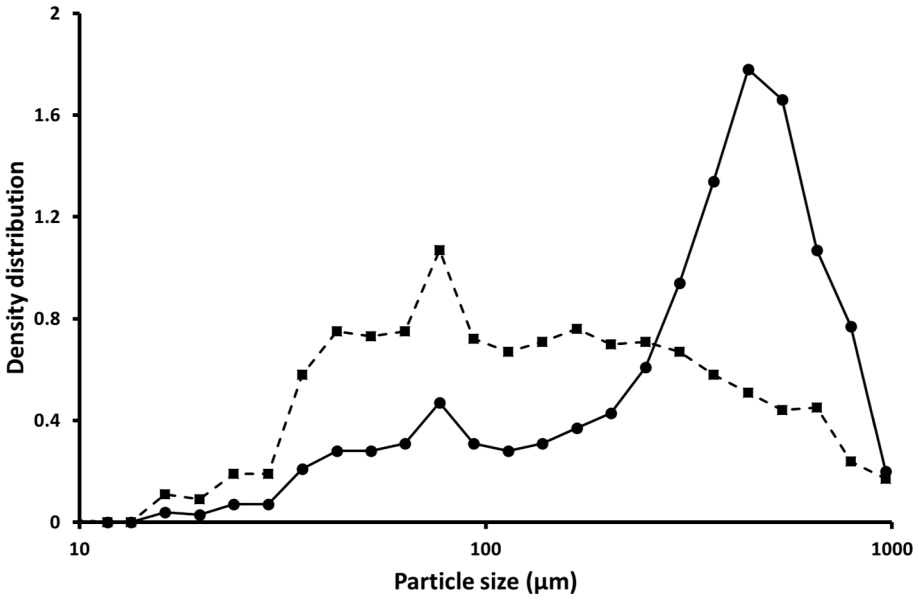

Fig. 1. Particle size distribution of standard milled and micro-milled flour. Samples of each flour were analysed by dynamic image analysis. Standard milled flour (circles and solid line) had a median particle size of 378 μm. Micro-milled flour (squares and dashed line) had a median particle size of 126 μm.

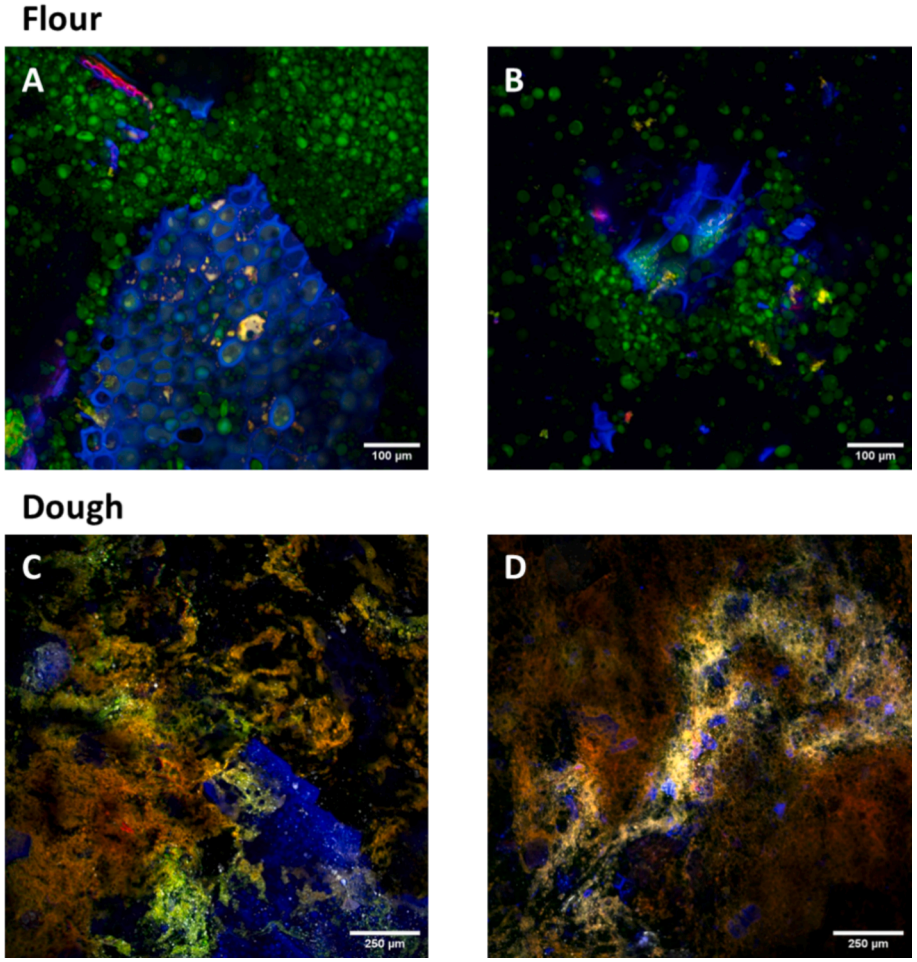

Fig. 2. Fluorescence imaging of wheat flour and doughs. Standard (A,C) and micro-milled (B,D) flour (A,B, ×63 magnification) and dough (C,D, ×20 magnification) samples stained with calcofluor white for cell walls (blue); fluorescein isothiocyanate (FITC) for starch (green); nile red for lipid (white); and fast green FCF for protein (red).

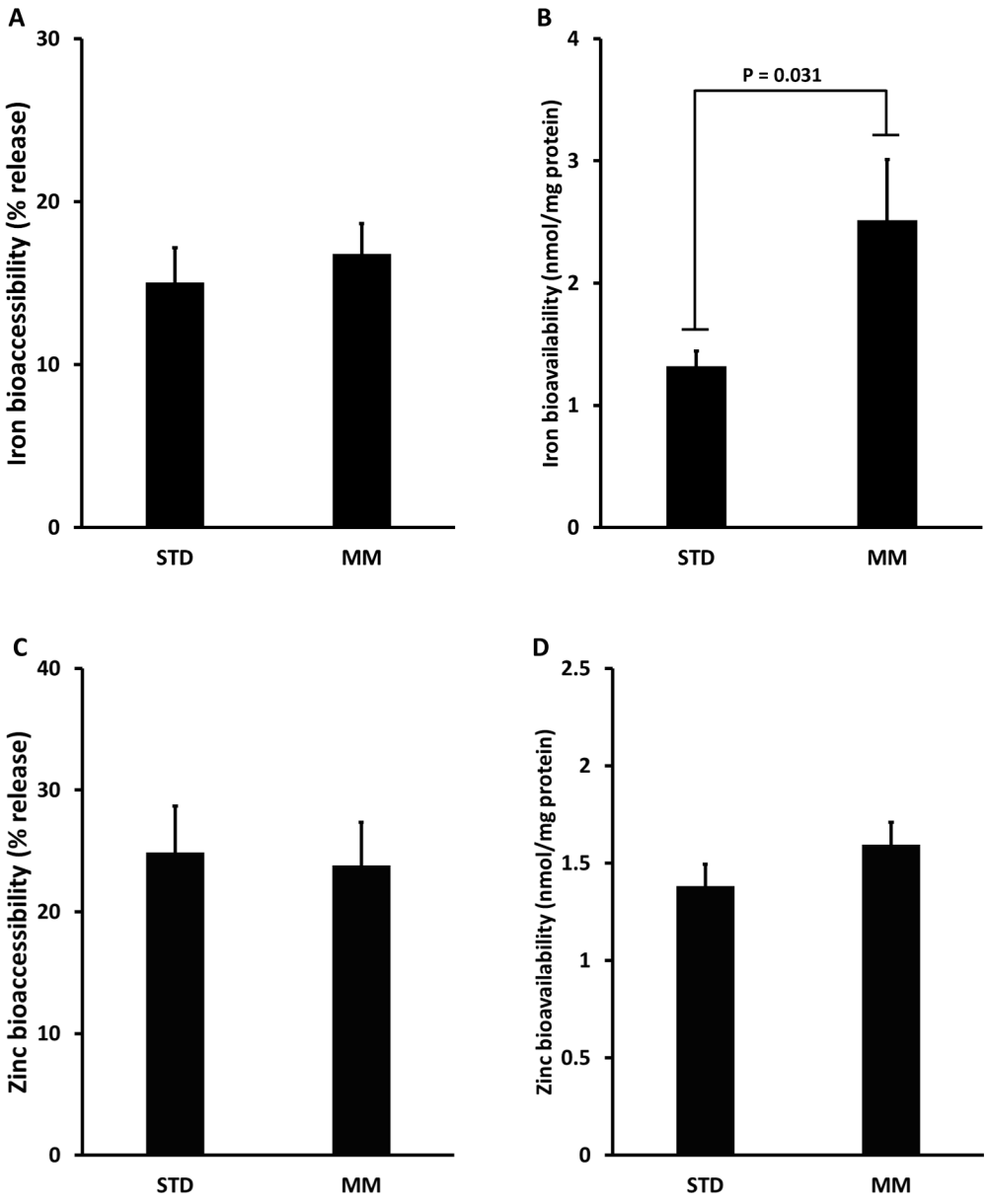

Fig. 3. Effect of flour type on mineral bioaccessibility and bioavailability from hydrothermally processed wheat doughs. Wheat doughs were produced using standard milled (STD) or micro-milled (MM) wholewheat flour and were boiled for 10 min. Iron (A), and zinc (C) bioaccessibility, measured as % of the initial iron content released from food following *in vitro* digestion, was not statistically different between flour types. Iron bioavailability (B), measured as the amount of iron from the *in vitro* digestate absorbed by Caco-2 cells, was significantly higher from MM flour doughs relative to STD flour doughs (P = 031, Student's unpaired *t*-test). There was no significant difference in zinc bioavailability (D) from wheat flour doughs between flour types. Data are means ± SEM, n = 5–6 (bioaccessibility) or 12–15 (bioavailability) independent measurements.

treatments, are known to be important determinants of nutrient bioaccessibility (Edwards et al., 2015a,2021; Grundy et al., 2016). In this study, micronization of flour reduced median particle size by two-thirds compared with standard roller-milled flour. Fluorescence imaging showed that the cellular structure of wholewheat flour and the corresponding wheat flour dough was disrupted by micro-milling and this corresponds with our previous work showing almost complete disruption of the aleurone cell walls in micronized flour (Latunde-Dada et al., 2014). We show here that micronization of flour and hydrothermal processing did not alter the iron content of flour or dough. However, there was a small but significant decrease in zinc content in the micro-milled flour and dough. This difference in mineral loss may reflect the relative localization of iron (predominantly in aleurone) and zinc (wider distribution including aleurone and embryo) in the wheat grain (Wan et al., 2022).

Hydrothermal processing of starchy foods such as wheat causes changes to food structure through swelling and gelatinisation of starch, and this in turn can impact nutrient digestion and/or absorption kinetics (Edwards et al., 2015b). To build on our previous work with uncooked wholewheat flour (Latunde-Dada et al., 2014), in the current study we prepared wheat flour doughs, subsequently cooked in boiling water, as simple model foods for analysis of mineral bioaccessibility and bioavailability. These doughs mimic real food systems, for example pasta and noodles, where the doughs are cooked in water. The cooked wheat products were subjected to *in vitro* gastrointestinal digestion to determine whether either hydrothermal processing or flour micronization (or a combination of the two) influenced mineral bioaccessibility. In our previous studies using uncooked wholewheat flour, iron bioaccessibility was significantly higher from the micronized flour (Latunde-Dada et al., 2014). In contrast, our current data found no

differences in the amount of iron or zinc liberated from the standard or micronized wheat flour products following *in vitro* digestion. This indicates that changes to food structure during cooking might impact mineral bioaccessibility.

Subsequently, digestion products from the hydrothermally processed wheat flour foods were added to human intestinal Caco-2 cells to assess iron and zinc bioavailability. Caco-2 cells are a well-established model for *in vitro* bioavailability studies and express the nutrient transporters required for the absorption of iron (reviewed in Sharp, 2005; Sandberg, 2010). For the boiled doughs, iron bioavailability was significantly higher from the micronized flour samples than from those produced using the standard milled flour. These findings agree with our previous work showing that physical disruption of wheat cell walls by micro-milling increases iron absorption from uncooked wheat flour (Latunde-Dada et al., 2014). Moreover, these studies demonstrate that bioaccessibility following *in vitro* digestion is a poor predictor of iron bioavailability. Multiple iron complexes with a wide range of particle sizes are likely to be released during digestion but not all will be available for intestinal absorption. While there is good evidence to support ionized ferrous iron absorption via DMT1 (Tandy et al., 2000; Sharp et al., 2002) and endocytic uptake of nanoparticulate iron in Caco-2 cells (Pereira et al., 2013; Perfecto et al., 2017), there is no evidence for uptake of larger iron complexes which might be liberated during *in vitro* digestion of wheat flour doughs. Our preliminary analysis using specific molecular weight filters showed that only 35.9 ± 6.4 % of iron released following *in vitro* digestion of the wheat flour doughs is in a low molecular weight form that can pass through a 3 kDa cut-off filter. This suggests that only a relatively small fraction of the bioaccessible iron is available for absorption.

It is estimated that up to 75 % of iron in wheat is contained within the aleurone layer where it localises with phosphorus, largely in multimeric complexes with the mineral absorption inhibitor phytic acid (Moore et al., 2012; Neal et al., 2013; De Brier et al., 2016; Wan et al., 2022). Several studies have shown that decreasing phytic acid content of wheat flour increases iron bioaccessibility and/or bioavailability (Brune, Rossander-Hultén, Hallberg, Gleerup, & Sandberg, 1992; Chaoui, Faid, & Belahsen, 2006; Sanz-Penella, Laparra, Sanz, & Haros, 2012; Rodriguez-Ramiro et al., 2017). It has been reported that phytic acid levels in flour can be modified by different milling methods (Antoine et al., 2003); however, in these studies we did not observe changes in phytate levels between the standard and micro-milled flours. Studies have reported that hydrothermal processing decreases the phytate content of legumes (Rehman & Shah, 2005). In the cooked doughs we also observed a decrease in phytate content (by approximately 50 %) compared with the raw flour, but this was not influenced by flour type. The phytate assay used in these studies only measures total phytate content and not the individual inositol phosphate metabolites (McKie & McCleary, 2016). Further analysis is warranted to determine whether the milling method might result in a shift from the strongly inhibitory IP_6 and IP_5 to the less inhibitory IP_4 and IP_3 metabolites.

In contrast to iron, zinc bioavailability was not altered significantly by flour micronization. While zinc is abundant in the phytate-rich aleurone it is also present at high levels in the wheat embryo (Wan et al., 2022). The effects of micronization and hydrothermal processing on the structure of the wheat germ was not investigated in this study, so it is not clear whether the embryo cell walls were disrupted to the same extent as the aleurone. Within the embryo, zinc is incorporated into several enzymes and proteins, which may in turn may allow association with endogenous wheat mineral chelators such as nicotianamine (Eagling et al., 2014; Beasley et al., 2019). The differences in tissue localisation and chemical speciation might protect zinc from the inhibitory effects of phytate and could explain, in part, the observations that zinc has higher bioavailability than iron in wheat products (Bouis & Welch, 2010), and why the mechanical and hydrothermal treatments reported here had only a limited impact on zinc bioavailability.

Taken together, our data demonstrate that physical disruption of wheat cell walls increases iron bioavailability from cooked wheat-based foods. Interestingly, we have shown previously that chemical degradation of cell walls using driselase (an enzyme mixture containing laminarinase, xylanase, and cellulase activity) also increases iron bioaccessibility from wheat flour (Latunde-Dada et al., 2014). This opens the possibility of using food-grade enzymes to improve iron bioavailability. For example, xylanases could be used to specifically target the arabinoxylan-rich aleurone cell walls to increase iron release from its major storage site in cereals. This approach has been shown to increase iron bioaccessibility from injera flours (Baye, Guyot, Icard-Vernière, Rochette, & Mouquet-Rivier, 2015) and could be applied to potentially increase iron bioaccessibility and bioavailability from other cereal foods.

5. Conclusions

Cereals and cereal products provide 50 % of the iron in the diet of UK adolescents, a group which is particularly prone to micronutrient deficiencies. The experimental strategies used in this study have the potential to increase the release and absorption of the endogenous iron present in cereal-based foods. This would not only enhance the nutritional quality of cereal products but would also have potential health benefits for population groups with poor iron status.

Author contributions

The project was conceived and supervised by PAS, GOL-D, SEB and PRE. Experimental work was performed by MFA, SMA and BB. Data were analysed by PAS, MFA and SMA. The manuscript was written by PAS, GOL-D and MFA and was reviewed and approved by all authors.

Funding

The work was funded by a project grant from the Biotechnology and Biological Sciences Research Council (BB/N021002). SMA was in receipt of a studentship funded by King Abdulaziz University, Jeddah, Saudi Arabia.

CRediT authorship contribution statement

Mohamad F. Aslam: Writing – review & editing, Methodology, Investigation, Formal analysis. **Sarah M. Arafsha:** Writing – review & editing, Methodology, Investigation. **Sarah E. Berry:** Writing – review & editing, Funding acquisition. **Balazs Bajka:** Writing – review & editing, Investigation, Formal analysis. **Peter R. Ellis:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Gladys O. Latunde-Dada:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Paul A. Sharp:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank Walter von Reding and Bühler AG, Uzwil, Switzerland, for providing the flour used in this study. We also thank Michael Adams and

Lucas Westphal, Campden BRI, Chipping Campden, UK, for proximate analysis and characterisation of wheat flour.

References

Antoine, C., Peyron, S., Mabille, F., Lapierre, C., Bouchet, B., Abecassis, J., & Rouau, X. (2003). Individual contribution of grain outer layers and their cell wall structure to the mechanical properties of wheat bran. *Journal of Agricultural and Food Chemistry, 51*(7), 2026–2033. https://doi.org/10.1021/jf0261598

Arafsha, S. M., Aslam, M. F., Ellis, P. R., Latunde-Dada, G. O., & Sharp, P. A. (2023). Strategies to increase the bioaccessibility and bioavailability of iron and zinc from cereal products. *Proceedings of the Nutrition Society*. https://doi.org/10.1017/S0029665123003543

Aslam, M. F., Ellis, P. R., Berry, S. E., Latunde-Dada, G. O., & Sharp, P. A. (2018). Enhancing mineral bioavailability from cereals: Current strategies and future perspectives. *Nutrition Bulletin, 43*(2), 184–188. https://doi.org/10.1111/nbu.12324

Balk, J., Connorton, J. M., Wan, Y., Lovegrove, A., Moore, K. L., Uauy, C., Sharp, P. A., & Shewry, P. R. (2019). Improving wheat as a source of iron and zinc for global nutrition. *Nutrition Bulletin, 44*(1), 53–59. https://doi.org/10.1111/nbu.12361

Baye, K., Guyot, J. P., Icard-Vernière, C., Rochette, I., & Mouquet-Rivier, C. (2015). Enzymatic degradation of phytate, polyphenols and dietary fibers in Ethiopian injera flours: Effect on iron bioaccessibility. *Food Chemistry, 174*, 60–67. https://doi.org/10.1016/j.foodchem.2014.11.012

Beasley, J. T., Bonneau, J. P., Sánchez-Palacios, J. T., Moreno-Moyano, L. T., Callahan, D. L., Tako, E., Glahn, R. P., Lombi, E., & Johnson, A. A. T. (2019). Metabolic engineering of bread wheat improves grain iron concentration and bioavailability. *Plant Biotechnology Journal, 17*(8), 1514–1526. https://doi.org/10.1111/pbi.13074

Bouis, H. E., & Welch, R. M. (2010). Biofortification-a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science, 50*, 20–32. https://doi.org/10.2135/cropsci2009.09.0531

Brnić, M., Wegmüller, R., Zeder, C., Senti, G., & Hurrell, R. F. (2014). Influence of phytase, EDTA, and polyphenols on zinc absorption in adults from porridges fortified with zinc sulfate or zinc oxide. *Journal of Nutrition, 144*(9), 1467–1473. https://doi.org/10.3945/jn.113.185322

Brouns, F., Hemery, Y., Price, R., & Anson, N. M. (2012). Wheat aleurone: Separation, composition, health aspects, and potential food use. *Critical Reviews in Food Science and Nutrition, 52*(6), 553–568. https://doi.org/10.1080/10408398.2011.589540

Brune, M., Rossander-Hultén, L., Hallberg, L., Gleerup, A., & Sandberg, A. S. (1992). Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. *Journal of Nutrition, 122*(3), 442–449. https://doi.org/10.1093/jn/122.3.442

Chaoui, A., Faid, M., & Belahsen, R. (2006). Making bread with sourdough improves iron bioavailability from reconstituted fortified wheat flour in mice. *Journal of Trace Elements in Medicine and Biology, 20*(4), 217–220. https://doi.org/10.1016/j.jtemb.2006.04.002

Christides, T., & Sharp, P. (2013). Sugars increase non-heme iron bioavailability in human epithelial intestinal and liver cells. *PLoS One., 8*(12), e83031. https://doi.org/10.1371/journal.pone.0083031

De Brier, N., Gomand, S. V., Donner, E., Paterson, D., Smolders, E., Delcour, J. A., & Lombi, E. (2016). Element distribution and iron speciation in mature wheat grains (*Triticum aestivum* L.) using synchrotron X-ray fluorescence microscopy mapping and X-ray absorption near-edge structure (XANES) imaging. *Plant Cell Environment, 39*(8), 1835–1847. https://doi.org/10.1111/pce.12749

Eagling, T., Neal, A. L., McGrath, S. P., Fairweather-Tait, S., Shewry, P. R., & Zhao, F. J. (2014). Distribution and speciation of iron and zinc in grain of two wheat genotypes. *Journal of Agricultural and Food Chemistry, 62*(3), 708–716. https://doi.org/10.1021/jf403331p

Edwards, C. H., Grundy, M. M., Grassby, T., Vasilopoulou, D., Frost, G. S., Butterworth, P. J., Berry, S. E., Sanderson, J., & Ellis, P. R. (2015a). Manipulation of starch bioaccessibility in wheat endosperm to regulate starch digestion, postprandial glycemia, insulinemia, and gut hormone responses: A randomized controlled trial in healthy ileostomy participants. *American Journal of Clinical Nutrition, 102*(4), 791–800. https://doi.org/10.3945/ajcn.114.106203

Edwards, C. H., Ryden, P., Mandalari, G., Butterworth, P. J., & Ellis, P. R. (2021). Structure-function studies of chickpea and durum wheat uncover mechanisms by which cell wall properties influence starch bioaccessibility. *Nature Food, 2*(2), 118–126. https://doi.org/10.1038/s43016-021-00230-y

Edwards, C. H., Warren, F. J., Campbell, G. M., Gaisford, S., Royall, P. G., Butterworth, P. J., & Ellis, P. R. (2015b). A study of starch gelatinisation behaviour in hydrothermally-processed plant food tissues and implications for in vitro digestibility. *Food and Function, 6*(12), 3634–3641. https://doi.org/10.1039/c5fo00754b

Grundy, M. M., Edwards, C. H., Mackie, A. R., Gidley, M. J., Butterworth, P. J., & Ellis, P. R. (2016). Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. *British Journal of Nutrition, 116*(5), 816–833. https://doi.org/10.1017/S0007114516002610

Hallberg, L., Brune, M., & Rossander, L. (1989). Iron absorption in man: Ascorbic acid and dose-dependent inhibition by phytate. *American Journal of Clinical Nutrition, 49*(1), 140–144. https://doi.org/10.1093/ajcn/49.1.140

Latunde-Dada, G. O., Li, X., Parodi, A., Edwards, C. H., Ellis, P. R., & Sharp, P. A. (2014). Micromilling enhances iron bioaccessibility from wholegrain wheat. *Journal of Agricultural and Food Chemistry, 62*(46), 11222–11227. https://doi.org/10.1021/jf503474f

McKie, V., & McCleary, B. V. (2016). A novel and rapid colorimetric method for measuring total phosphorus and phytic acid in foods and animal feeds. *Journal of AOAC International, 99*, 3. https://doi.org/10.5740/jaoacint.16-0029

Moore, K. L., Zhao, F.-J., Gritsch, C., Tosi, P., Hawkesford, M. J., McGrath, S. P., Shewry, P. R., & Grovenor, C. R. M. (2012). Localisation of iron in wheat grain using high resolution secondary ion mass spectrometry. *Journal of Cereal Science, 55*, 183–187. https://doi.org/10.1016/j.jcs.2011.11.005

National Diet and Nutrition Survey: Years 9 to 11 of the Rolling Programme (2016/2017 to 2018/2019). <https://www.gov.uk/government/statistics/ndns-results-from-years-9-to-11-2016-to-2017-and-2018-to-2019> Accessed April 2024.

Neal, A. L., Geraki, K., Borg, S., Quinn, P., Mosselmans, J. F., Brinch-Pedersen, H., & Shewry, P. R. (2013). Iron and zinc complexation in wild-type and ferritin-expressing wheat grain: Implications for mineral transport into developing grain. *Journal of Biological and Inorganic Chemistry, 18*(5), 557–570. https://doi.org/10.1007/s00775-013-1000-x

Perfecto, A., Elgy, C., Valsami-Jones, E., Sharp, P., Hilty, F., & Fairweather-Tait, S. (2017). Mechanisms of iron uptake from ferric phosphate nanoparticles in human intestinal Caco-2 cells. *Nutrients, 9*(4), 359. https://doi.org/10.3390/nu9040359

Pereira, D. I., Mergler, B. I., Faria, N., Bruggraber, S. F., Aslam, M. F., Poots, L. K., Prassmayer, L., Lönnerdal, B., Brown, A. P., & Powell, J. J. (2013). Caco-2 cell acquisition of dietary iron(III) invokes a nanoparticulate endocytic pathway. *PLoS One, 8*(11), Article e81250. https://doi.org/10.1371/journal.pone.0081250

Rehman, Z., & Shah, W. H. (2005). Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chemistry, 91*, 327–331. https://doi.org/10.1016/j.foodchem.2004.06.019

Rodriguez-Ramiro, I., Brearley, C. A., Bruggraber, S. F., Perfecto, A., Shewry, P., & Fairweather-Tait, S. (2017). Assessment of iron bioavailability from different bread making processes using an in vitro intestinal cell model. *Food Chemistry, 228*, 91–98. https://doi.org/10.1016/j.foodchem.2017.01.130

Sandberg, A. S. (2010). The use of Caco-2 cells to estimate Fe absorption in humans – A critical appraisal. *International Journal of Vitamin and Nutrition Research, 80*(4–5), 307–313. https://doi.org/10.1024/0300-9831/a000038

Sanz-Penella, J. M., Laparra, J. M., Sanz, Y., & Haros, M. (2012). Assessment of iron bioavailability in whole wheat bread by addition of phytase-producing bifidobacteria. *Journal of Agricultural and Food Chemistry, 60*(12), 3190–3195. https://doi.org/10.1021/jf205048r

Sharp, P., Tandy, S., Yamaji, S., Tennant, J., Williams, M., & Srai, S. K. S. (2002). Rapid regulation of divalent metal transporter (DMT1) protein but not mRNA expression by non-haem iron in human intestinal Caco-2 cells. *FEBS Letters, 510*(1–2), 71–76. https://doi.org/10.1016/s0014-5793(01)03225-2

Sharp, P. (2005). Methods and options for estimating iron and zinc bioavailability using Caco-2 cell models: Benefits and limitations. *International Journal of Vitamin and Nutrition Research, 75*(6), 413–421. https://doi.org/10.1024/0300-9831.75.6.413

Sreenivasulu, K., Raghu, P., Ravinder, P., & Nair, K. M. (2008). Effect of dietary ligands and food matrices on zinc uptake in Caco-2 cells: Implications in assessing zinc bioavailability. *Journal of Agricultural and Food Chemistry, 56*(22), 10967–10972. https://doi.org/10.1021/jf802060q

Tandy, S., Williams, M., Leggett, A., Lopez-Jimenez, M., Dedes, M., Ramesh, B., Srai, S. K., & Sharp, P. (2000). Nramp2 expression is associated with pH-dependent iron uptake across the apical membrane of human intestinal Caco-2 cells. *Journal of Biological Chemistry, 275*(2), 1023–1029. https://doi.org/10.1074/jbc.275.2.1023

Wan, Y., Stewart, T., Amrahli, M., Evans, J., Sharp, P., Govindan, V., Hawkesford, M. J., & Shewry, P. R. (2022). Localisation of iron and zinc in grain of biofortified wheat. *Journal of Cereal Science, 105*, Article 103470. https://doi.org/10.1016/j.jcs.2022.103470