Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2025, **16**, 9377

Zinc absorption from breakfast flakes produced from sprouted or hydrothermally processed wheat: a randomized cross-over human intervention study

Marie Huyskens, *[a] Elien Lemmens, [b] Lana Abou-Zeid, [c] Kasper Hobin, [c] Lukas M. Balsiger, [d] Frank Vanhaecke, [c] Kristin Verbeke, [d] Erik Smolders [e] and Jan A. Delcour [a]

Zinc (Zn) deficiency is a health issue worldwide. Although wheat is a valuable source of dietary Zn, its bioaccessibility is limited due to chelation with phytic acid. Steeping wheat at 15 °C for 36 h followed by sprouting at 26 °C for 48 h led to 26% phytate reduction and a 1.4-fold increase in Zn bioaccessibility. Hydrothermal processing of wheat at 60 °C and pH 4.0 for 24 h in a 0.1 M sodium citrate buffer reduced phytate content by 48% and increased Zn bioaccessibility 9.9-fold. Here, the potential of sprouting or hydrothermal processing of wheat to increase Zn absorption was studied in a human intervention study using a dual isotope technique in which breakfast flakes were extrinsically labeled with enriched stable isotopes of Zn. One group of participants consumed flakes from untreated and sprouted wheat (test group I, n = 24), whereas another group (test group II, n = 12) consumed flakes from untreated and hydrothermally treated wheat. Sprouting of wheat did not significantly increase fractional Zn absorption, while hydrothermal processing of wheat resulted in a 1.1-fold increase in fractional Zn absorption compared to untreated wheat. The modest if any increase in fractional Zn absorption resulting from sprouting or hydrothermal processing of wheat was likely due to high residual phytate : Zn molar ratios. These findings show that assessing phytate reduction and Zn bioaccessibility values of food products alone is not sufficient to predict Zn absorption from food products.

Received 27th August 2025,
Accepted 5th November 2025

DOI: 10.1039/d5fo03650j

rsc.li/food-function

1. Introduction

With an estimated production of 788 million tons in 2024, wheat is the second most-produced cereal worldwide.[1,2] The majority of wheat-based food products are made from white flour which is produced by removing the micronutrient-rich bran layers from the energy-dense but micronutrient-poor endosperm.[3] This is unfortunate because the consumption of whole grain products has been associated with reduced risk of diet-related diseases such as obesity, cardiovascular diseases, type II diabetes, and certain cancers.[4] The reduced disease risks can be attributed to the high amount of dietary fiber and bioactive compounds, including polyphenols, B vitamins, and minerals like iron (Fe) and zinc (Zn) in the bran fraction.[4,5]

The aleurone layer of the miller's bran is a good source of Zn and accounts for most of the Zn in whole wheat (20–60 mg kg^{-1}).[6] Cereal products, which are mainly wheat-based, provide 25–31% of the average Zn intake in the UK.[7] As Zn is an essential cofactor for more than 200 enzymes in the body,[8] adequate intake is crucial *e.g.* for child growth, neurobehavioral development, and well-functioning of the immune system.[9]

The average daily Zn requirement ranges from 6 to 13 mg.[10] For Zn ions to be absorbed in the intestinal lumen and utilized by the human body, they first need to be released from their matrix into the gastrointestinal tract, *i.e.* they need to be bioaccessible.[11,12] Unfortunately, Zn in wheat has only limited bioaccessibility (3–5%)[13] because it is chelated by phytic acid (*i.e.* myo-inositol 1,2,3,4,5,6-hexakisphosphate, IP_6), the main storage form of phosphorus (P) in cereals.[14] The chelates (*i.e.*

[a]*Laboratory of Food Chemistry and Biochemistry, KU Leuven, Belgium. E-mail: marie.huyskens@kuleuven.be, jan.delcour@kuleuven.be; Tel: (+32)-16-194978*
[b]*Center for Sustainable Catalysis and Engineering, KU Leuven, Belgium. E-mail: elien.lemmens1@kuleuven.be*
[c]*Atomic and Mass Spectrometry – A&MS research unit, Department of Chemistry, Ghent University, Belgium. E-mail: lana.abouzeid@ugent.be, kasper.hobin@ugent.be, frank.vanhaecke@ugent.be*
[d]*Translational Research Center for Gastrointestinal Disorders, KU Leuven, Belgium. E-mail: lukasmichaja.balsiger@kuleuven.be, kristin.verbeke@kuleuven.be*
[e]*Division of Soil and Water Management, KU Leuven, Belgium. E-mail: erik.smolders@kuleuven.be*

phytates) are insoluble at intestinal pH,[15] and can be, in principle, degraded by phytases.[16] However, the human enzyme arsenal contains only limited amounts of these enzymes.[17,18] The impact of phytate in cereal-based products on Zn bioaccessibility and absorption has been extensively investigated.[19–23]

Dephytinization techniques aimed at increasing mineral bioaccessibility in food products have recently been thoroughly discussed by El Houssni *et al.*[24] One such technique is cereal sprouting, *i.e.* the process that initiates seedling growth once environmental conditions such as temperature and moisture content are favorable. For sprouting to occur, grains need to absorb water during a steeping step. The water uptake activates their metabolism and boosts respiration.[25] During subsequent sprouting, various enzymes (including phytases) are activated and/or *de novo* synthesized.[26] Lemmens *et al.*[27] demonstrated that sprouting wheat at 26 °C for 120 h reduced phytate content by 33% and increased Zn bioaccessibility readings from 3% to 21%. Another dephytinization process is hydrothermal treatment. Indeed, incubating wheat grains for several hours at optimal conditions for phytase activity (50–60 °C; pH 3.0–4.0) results in substantial phytate degradation.[13] Additionally, incubation of grains with some aqueous buffered media impacts Zn bioaccessibility by forming soluble Zn-complexes.[22] However, to this day, it remains unclear whether food products made from sprouted or hydrothermally processed wheat effectively result in increased Zn absorption in the human body.

In this work, breakfast flakes were produced from untreated, sprouted, and hydrothermally processed wheat. The phytate contents and Zn bioaccessibility were investigated, and the Zn absorption from the breakfast flakes was quantified *in vivo* in a human intervention study with two separate groups using a dual isotope technique.

To measure Zn absorption, each type of breakfast flakes was extrinsically labeled with an enriched stable isotope of Zn. Enriched stable isotopes are commonly used in the research field, as their absorption closely reflects that of native Zn and can be accurately quantified.[28] Zinc has three stable isotopes, ^{67}Zn, ^{68}Zn, and ^{70}Zn, that can be used as an isotopically enriched tracer in *in vivo* studies because of their low natural abundance (*i.e.* 4.04%, 18.45%, and 0.61%, respectively).[28,29]

The dual isotope technique to measure Zn absorption was introduced by Friel *et al.*[30] and involves, in addition to the consumption of isotopically labeled (^{67}Zn and ^{68}Zn) breakfast flakes, also the intravenous administration of ^{70}Zn. The ^{70}Zn serves as a systemic reference tracer and allows to correct for individual differences in the Zn distribution pool, excretion, and isotope recovery. By comparing the relative abundances of the oral (^{67}Zn and ^{68}Zn) and intravenous (^{70}Zn) tracers in urine, the fractional absorption of the oral Zn dose can be estimated assuming complete isotopic equilibration and similar renal handling of both isotopes.[30] Measurement of isotope ratios in urine obviates the need for blood sampling, rendering the procedure more convenient for the participants.[30,31] Because of the need for one Zn isotope as intravenous reference, and the long biological half-life of Zn, which prevents repetitive administration of the same isotope to individuals,[32] only two types of breakfast flakes, labeled with ^{67}Zn and ^{68}Zn, respectively, could be tested by the same individuals. Therefore, one group of participants consumed flakes from untreated and sprouted wheat (test group I), whereas another group (test group II) consumed flakes from untreated and hydrothermally treated wheat.

To the best of the authors' knowledge, theirs is the first effort to investigate Zn absorption in healthy participants from a food product made from solely sprouted or hydrothermally processed wheat.

2. Materials and methods

2.1 Materials

Winter wheat cultivar LG Skyscraper was obtained from Limagrain (Avelgem, Belgium). Concentrated hydrochloric acid (HCl) and nitric acid (HNO_3) were from NORMATOM (VWR Chemicals, Leuven, Belgium). The certified reference wheat flour sample (SRM 1567b) was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). The certified reference samples SRM 1643f (Trace Element in Water) was from NIST, and SPS-SW2 Batch 137 (Reference Material for Measurement of Elements in Surface Waters) was from Spectrapure Standards (Oslo, Norway). Multi-element solutions with certified element compositions were from Certipur Merck (Darmstadt, Germany). Digestive enzymes [pepsin (P-7012) and pancreatin (P-3292) preparations] were purchased from Sigma-Aldrich (Bornem, Belgium), as was Ultrapure TraceSELECT hydrogen peroxide (H_2O_2; 30% w/w). Zinc oxides highly enriched in ^{67}Zn, ^{68}Zn, or ^{70}Zn (enrichments exceeding 89%, 98%, and 98%, respectively) were purchased from Isoflex (San Francisco, CA, USA). In these products, the concentrations of other mineral elements were lower than 300 ppm. Ultrapure water (18.2 MΩ cm) was produced with a Merck Milli-Q IQ Element water purification & dispensing unit. Trace metal grade concentrated HNO_3 and HCl used during preparation of the urine samples (see 2.5.1) were from Fisher Chemicals (Leicestershire, UK) and further purified by sub-boiling distillation with a Savillex (Eden Prairie, MN, USA) DST-4000 acid purification system. Zinc was isolated chromatographically from the urine samples with Eichrom Technologies (Lisle, IL, USA) polypropylene columns (internal diameter 8 mm) loaded with AG MP-1 M anion exchange resin (100–200 μm dry mesh size, chloride anionic form) from Bio-Rad (Lokeren, Belgium). The urine reference material Seronorm Trace Elements Urine L-1 (Sero, Billingstad, Norway, lot 1706877) was prepared according to Seronorm's guidelines. Isotopic reference material IRMM-3702 was obtained from the Institute of Reference Materials and Measurements (Geel, Belgium). A single-element standard stock solution of Zn and copper (Cu) was obtained from Inorganic Ventures (Christiansburg, VA, USA). Unless stated otherwise, all other used chemicals were of at least analytical grade and from Merck.

2.2 Breakfast flakes preparation and characterization

2.2.1 Sprouting and hydrothermal processing of wheat grains. Wheat grain batches (5.5 kg each) were steeped and sprouted using a pilot-scale micro-malting device (Joe White Malting Systems, Perth, Australia). The steeping process consisted of three wet stages of 8 h at 15 °C, alternated by two intermediate air rest stages of 6 h at 15 °C. Steeping of the grains was done in tap water. The steeped grains were then sprouted in the same device at 26 °C for 48 h. The air rest stages during the steeping process and the sprouting process were done with an airflow of 175 L min^{-1} and 50% recirculation.

For hydrothermal processing, non-sprouted wheat grain batches (0.5 kg each) were incubated at 60 °C and pH 4.0 for 24 h in 1.0 L 0.1 M sodium citrate buffer as described in Huyskens *et al.*[22] After incubation, the grains were rinsed three times with demi-water to remove residual acid from their surface.

Both sprouted and hydrothermally processed wheat grains were autoclaved at 121 °C for 10 min and oven-dried at 60 °C to moisture contents in a 9 to 3% range. Both types of processed wheat and the starting material were then milled with a FOSS Tecator (Hillerød, Denmark) Cyclotec 1093 sample mill into whole meal (<500 μm) for breakfast flake production.

2.2.2 Production of breakfast flakes. The three types of breakfast flakes were made on pilot-scale at Dailycer France (Faverolles, France) with a twin screw APV Baker (Baker Perkins, Peterborough UK) extruder. Whole meal water suspension was extruded at 100 °C and 41 bar at a screw speed of 175 rotations per min (rpm) and a nozzle diameter of 4 mm. At the die, the extrudates were cut into pellets with a moisture content in an 18 to 27% range, and a production flow rate of 67 kg h^{-1}. These pellets were then flaked at room temperature between rolls and toasted using a Jetzon Jettube Dryer (Wolverine, Methuen, MA, USA) roaster at 195 °C for 90 s, resulting in flakes with a moisture content below 4.0%.

2.2.3 Analysis of breakfast flakes. The phytate content [g per 100 g dry matter (dm)] of the breakfast flakes was determined in triplicate from the P concentrations in acid extracts before (*i.e.* free P) and after enzymatic phytate hydrolysis (*i.e.* total P) using the Megazyme (Bray, Ireland) K-Phyt assay, as described in Huyskens *et al.*[22]

The bioaccessibility of Zn in the flakes was evaluated in triplicate using an *in vitro* digestion, as described in Huyskens *et al.*[22] This procedure mimics *in vivo* gastrointestinal conditions by introducing specific digestive enzymes (*i.e.* pepsin in the gastric phase and pancreatin in the intestinal phase) in suspensions of breakfast flakes and incubating them at 37 °C and pH 2.0 for the gastric phase and pH 6.8 for the intestinal phase. Following *in vitro* digestion, the suspensions were centrifuged (10 000*g*, 15 min, room temperature) and filtered (Whatman filter, GE Healthcare Life Sciences, Buckinghamshire, UK) to separate the supernatant (*i.e.* digested fraction) from the residue (*i.e.* undigested fraction). The mineral concentrations in the breakfast flakes and in the *in vitro* digested and undigested fractions were determined with inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700×, Agilent Technologies, Santa Clara, CA, USA) as described in Huyskens *et al.*[22] The limit of quantification for Zn was 0.23 mg kg^{-1}.

The bioaccessibility of Zn in the breakfast flakes was the percentage of the total Zn content in the flakes which ended up in the supernatant (*i.e.* the digested fraction). The sums of the Zn contents in the *in vitro* digested and undigested fractions deviated by no more than 10% from the contents in the breakfast flakes.

2.3 Preparation of the enriched stable isotope solutions for oral and intravenous administration

Enriched Zn isotopes for oral administration (^{67}Zn and ^{68}Zn) were prepared by diluting the respective isotope oxides in excess 0.5 M H_2SO_4. Dilution with commercial bottled water (Spa Reine, Spa, Belgium) resulted in enriched stable isotope solutions containing 16 mg L^{-1} isotope. A residual concentration of 0.12 mM of H_2SO_4 was found in the enriched isotope solutions. The ^{70}Zn solutions for intravenous administration (22 μg g^{-1} of ^{70}Zn in saline solution) were prepared at the Cantonal Pharmacy of the University Hospital Zurich as described in Wegmüller *et al.*[33]

2.4 Human intervention study

2.4.1 Participants. Healthy participants were recruited *via* flyers spread around KU Leuven campuses. The inclusion criteria were: age between 18 and 50 years and a body mass index between 18.5 and 25.0 kg m^{-2}. None of the participants took vitamin or mineral supplements or medicines with an impact on the gastrointestinal tract in the period from two weeks before the start of the study until sampling was completed. Individuals who took antibiotics in the three months prior to the study were excluded. Other exclusion criteria were: previous or current gastrointestinal (*e.g.* Crohn's disease), endocrine or eating disorders or other gastrointestinal histories, previous or current substance/alcohol dependence or abuse (more than 2 units per day or 14 units per week), current smoking (having smoked in the last 28 days) or willingness to smoke during the study period, pregnancy or lactating or wishing to become pregnant, allergy or intolerance to wheat or citric acid, adherence to vegan or vegetarian diets or special diets, blood donation in the 6 months prior to the study.

2.4.2 Extrinsic labeling of breakfast flakes. The different types of breakfast flakes were labeled extrinsically with different enriched stable isotopes of Zn. Extrinsic labeling is based on the assumption that the extrinsic label completely equilibrates in the stomach with the intrinsic (unlabeled) Zn of the food or meal.[34,35] Due to the low pH in the stomach, minerals are released as free cations, allowing exchange. In the duodenum, they are again chelated by phytic acid, if present.[36] This assumption of equilibration of extrinsic Zn and wheat Zn in the stomach was evaluated using *in vitro* digestion. Breakfast flakes extrinsically labeled with ^{70}Zn were subjected to *in vitro* digestion mimicking the stomach phase as

described in Huyskens *et al.*[22] The soluble fraction was collected, and the isotope ratio (^{70}Zn/total Zn in soluble fraction) was measured and compared to the ratio in the extrinsically labeled breakfast flakes (^{70}Zn/total Zn in breakfast flakes). Complete isotopic equilibration between the extrinsic label and all forms of Zn in the flakes resulted in an equal isotope ratio in both samples. Based on the results obtained (SI, Table S1), it was concluded that the labeling approach used was justified. Similarly, Brnić *et al.*[37] and Signorell *et al.*[38] showed that extrinsic labels of Zn isotopes are absorbed to a similar extent as Zn in rice or wheat, respectively, showcasing the validity of the method.

2.4.3 Study design. The double-blind, randomized, cross-over design was approved by the Ethics Committee Research UZ Leuven/KU Leuven (S64419) in compliance with the latest version of the Declaration of Helsinki and the Belgian law of May 7, 2004 on experiments on the human person. The study was registered at clinicaltrials.gov (NCT06236620). All participants provided written informed consent. The screening and study visits took place between March 26 and July 2, 2024. The study took 8 days. An online randomization software (https://www.randomizer.org) was used to allocate the participants to one of the two test groups, to allocate a random code to each participant and each type of breakfast flakes, and to randomize the order of consumption of the breakfast flakes.

The participants collected a midstream urine sample on the morning of the first test day at home in a sterile, Zn-free plastic container. Afterwards, they came fasted to the laboratory where a ^{70}Zn stable isotope (0.22 mg) was injected intravenously into the antecubital vein over 10 minutes through an indwelling intravenous catheter. Within 30 minutes after intravenous administration, the participants consumed a breakfast meal consisting of 100 g flakes and 50 mL of specific enriched stable isotope solution (16 mg L^{-1}). Group I participants consumed regular breakfast flakes labeled with ^{67}Zn or flakes produced from sprouted wheat labeled with ^{68}Zn on two consecutive mornings in random order. Group II participants consumed regular breakfast flakes labeled with ^{67}Zn or flakes derived from hydrothermally processed wheat labeled with ^{68}Zn, also on two consecutive mornings in random order.

To ensure complete ingestion of the isotope solution, participants rinsed the cup of the enriched stable isotope solution three times with 50 mL water and drank the rinsing water along with the meal. After consumption of either the regular breakfast flakes or the flakes prepared from either sprouted or hydrothermally processed wheat, they received standard lunch and dinner meals for consumption on the same day. Besides that, they were only allowed to drink water. The breakfast flakes for the second test day as well as the specific Zn isotope solution were provided to the participants for consumption at home.

Participants also collected midstream urine samples in the morning at home on days 5, 6, 7, and 8 as described above and stored them at −20 °C. They were asked to refrain from excessive exercise one day prior to the experiment and on test days 1 and 2. Any adverse events they experienced (related or unrelated to the intervention) during the entire study period were reported.

2.4.4 Sample size calculation. To determine the number of participants needed to allocate potential statistical differences in fractional Zn absorption (see 2.5.3) between the regular breakfast flakes and those derived from sprouted or hydrothermally processed wheat, a power calculation (95% power), based on a two-tailed paired *t*-test, was made with the freeware G*Power 3.1.9.7. This power calculation was performed using expected fractional Zn absorption values for the breakfast flakes (SI, Table S2), based on Caco-2 Zn bioavailability data,[22,39] which were rescaled using available *in vivo* fractional Zn absorption values.[40] The power calculation made use of the expected interindividual difference in fractional Zn absorption of 10% (stdev) noted by Brnić *et al.*[37] and an estimated correlation of 0.3 between the fractional Zn absorption of the different breakfast flakes within a same participant. A dropout rate of 20% was used. To detect a minimum difference of 3.7% in fractional Zn absorption between regular wheat flakes and flakes derived from sprouted wheat, and a difference of 11.5% between regular wheat flakes and those made from hydrothermally processed wheat, 24 and 12 participants were needed, respectively (SI, Table S2).

2.5 Isotopic analysis of urine samples

2.5.1 Sample preparation. The sample preparation was carried out in a class-10 clean laboratory. Urine samples (2.00 mL) were accurately pipetted in pre-cleaned Teflon Savillex® beakers. The samples were acid digested by adding 2.0 mL of 14.0 M HNO_3 and 0.5 mL of 9.8 M H_2O_2 and heating the closed beakers on a hotplate at 110 °C for 16 h. Prior to the chromatographic isolation of Zn, the acid digests were evaporated to dryness at 90 °C, followed by dissolution in 5.0 mL of 8.0 M HCl containing 0.001% H_2O_2. The chromatographic isolation of Zn was carried out as outlined in Van Heghe *et al.*[41] Briefly, polypropylene columns filled with 1.0 mL of AG® MP-1 M strong anion exchange resin were cleaned with 10 mL of 7.0 M HNO_3, followed by 10 mL of MQ-water, 10 mL of 0.7 M HNO_3, and 10 mL of MQ-water. The columns were then conditioned with 10 mL of 8.0 M HCl containing 0.001% H_2O_2 and loaded with the samples dissolved in 5.0 mL of the same solution. Elution of the matrix was accomplished with 3.0 mL of 8.0 M HCl containing 0.001% H_2O_2, and the Cu and Fe ions were eluted with 9.0 mL of 5.0 M HCl containing 0.001% H_2O_2 and 7.0 mL of 0.53 M HCl, respectively. Finally, the Zn fraction was collected by adding 7.0 mL of 0.7 M HNO_3. The fractions containing Zn were evaporated to dryness and redissolved in 0.5 mL of 0.28 M HNO_3. Two procedural blanks were treated identically. The maximum blank contribution was 1.8% of the Zn concentration in the urine samples. Finally, the Zn fractions, the isotopic reference material IRMM-3702, and the single-element Zn standard stock solution were all diluted to 200 ng mL^{-1}. The latter is hereafter referred to as Zn in-house standard. Finally, Cu in-house standard was added to all samples to a concentration of 200 ng mL^{-1} as an internal standard relied

on for correction of the mass bias induced by instrumental mass discrimination.

2.5.2 Instrumentation and measurements. The Zn isotope ratios in the isolated Zn fractions were determined using a Neptune *Plus* Multicollector Inductively Coupled Plasma-Mass Spectrometer (MC-ICP-MS, Thermo Scientific, Bremen, Germany) equipped with a Jet interface (Jet-type Ni sampling cone and X-type Ni skimmer cone) for higher sensitivity. To monitor the Zn isotopes on the interference-free shoulder of the spectral peak, the measurements were performed at (pseudo) medium resolution. The instrument settings, data acquisition parameters, and internal precision obtained during isotope ratio measurements are summarized in the SI (Tables S3 and S4). Mass bias correction was done relying on a combination of internal and external corrections. The first approach was through internal normalization using Cu as internal standard. This correction was based on the so-called Baxter approach.[42] External correction was based on a standard solution (isotopic reference material IRMM-3072) measured in a sample-standard bracketing approach. The standard solution was measured before and after each sample, and it was assumed that mass bias drift (if any) was linear.

Zinc isotope ratios were calculated as follows:

$$\delta^{x}\mathrm{Zn}() = \left[\frac{(\delta^{x}\mathrm{Zn}/\delta^{64}\mathrm{Zn})_{\mathrm{sample}}}{(\delta^{x}\mathrm{Zn}/\delta^{64}\mathrm{Zn})_{\mathrm{IRMM\text{-}3072}}} - 1\right] \times 1000$$

with x = 67, 68 or 70.

Furthermore, the Zn in-house solution (with a previously characterized isotopic signature) was used for quality control at the beginning of and during every measurement session. The accuracy of the sample preparation and MC-ICP-MS measurement was evaluated by including Seronorm™ Trace Elements Urine L-1 in each batch of samples. The average isotope ratio (δ^{66}Zn) obtained for this reference sample was −0.13 ± 0.10 ‰, and was in agreement with data by Moore *et al.*[43,44]

2.5.3 Calculation of fractional and total Zn absorption. The enrichment of the isotopes administered orally and intravenously, which declined exponentially over time,[31] were plotted for each data set. The dual isotope technique assumes that the intravenous and oral doses are administered simultaneously.[45] However, since two types of flakes were tested, the intravenous dose could only be administered simultaneously with the consumption of one type. To correct for the delay in consuming the second type of flakes on the following day, the exponential decay function of the orally administered isotope on the second day was time-shifted by 24 h to the intravenous isotope curve.[30]

As also done by Dias *et al.*,[46] fractional Zn absorption of each type of breakfast flakes was calculated using the following equation:

$$\text{fractional Zn absorption} = \frac{n_{\mathrm{oral}}}{n_{\mathrm{iv}}} \times \frac{d_{\mathrm{iv}}}{d_{\mathrm{oral}}} \times 100$$

with n_{oral} and n_{iv} the enrichments of orally (^{67}Zn or ^{68}Zn) and intravenously (^{70}Zn) administered isotopic tracers in the urine sample, respectively, and d_{iv} and d_{oral} the doses of intravenously (0.22 mg) and orally administered tracers (0.8 mg).[30] The oral-to-intravenous stable isotope ratios ($n_{\mathrm{oral}}/n_{\mathrm{iv}}$) were calculated from the measured isotope ratios (^{67}Zn/^{64}Zn and ^{68}Zn/^{64}Zn, and ^{70}Zn/^{64}Zn). Since the ratio of oral to intravenously administered tracers becomes stable with time,[30] the mean of the fractional Zn absorption values of the four urine samples (days 5–8) was used.

The total Zn absorption (mg per portion) from the breakfast flakes was calculated by multiplying the total Zn content of the meal by the fractional Zn absorption.

2.6 Statistical analyses

JMP Pro 17 (SAS Institute, Cary, NC, USA) was used to trace down significant differences (p-value <0.05) in mean values of responses in the study (*e.g.* phytate contents, Zn contents, Zn bioaccessibility) among treatments, and a one-way ANOVA with Tukey multiple comparison procedure was performed. The mean of the fractional Zn absorption values of the four urine samples of the same test persons (days 5–8) is given. The fractional and total Zn absorption data were weighted using the reciprocal of the variance of the data point. A two-sided paired t-test was executed to determine whether the mean values of fractional and total Zn absorption were higher for flakes derived from either sprouted or hydrothermally processed wheat compared to regular flakes. The level of statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1 Phytate content and Zn bioaccessibility of breakfast flakes derived from untreated, sprouted, and hydrothermally processed wheat

The phytate content of (regular) flakes derived from untreated wheat (0.87 g per 100 g dm, Table 1) was in line with values for untreated wheat reported by Reddy *et al.*[47] The phytate : Zn molar ratio in regular flakes was 35.0. Breakfast flakes derived from steeped (15 °C, 36 h) and sprouted (26 °C, 48 h) wheat had a 26% lower phytate content and a reduced phytate : Zn molar ratio of 19.8. Earlier, Bartnik & Szafrańska[48] and Lemmens *et al.*[27] reported 18% and 32% phytate reduction when wheat was sprouted for 48 h at 20 °C and 26 °C, respectively.

Breakfast flakes made from wheat hydrothermally processed at 60 °C and pH 4.0 for 24 h in 0.1 M sodium citrate buffer had a 48% lower phytate content than flakes made from untreated wheat, and a phytate : Zn molar ratio 23.9 (Table 1). This was expected based on phytate contents of hydrothermally processed wheat reported by Huyskens *et al.*[22] Earlier, Fredlund *et al.*[49] observed 46% phytate reduction when wheat was hydrothermally processed (55 °C, 24 h). When an acetate buffer (pH 4.8, unspecified cation and concentration) was used under the same conditions, the phytate content was

Table 1 Phytate contents [g per 100 g dry matter (dm)], zinc (Zn) bioaccessibility (% of total Zn in wheat), total Zn content (mg kg^{-1} dm) and molar phytate : Zn ratio of breakfast flakes made from untreated wheat, steeped (15 °C, 36 h) and sprouted (26 °C, 48 h) wheat, and hydrothermally processed (60 °C, pH 4.0, 24 h) wheat using a 0.1 M sodium citrate buffer. Means and standard deviations of three technical repeats are given. Mean values within a parameter (*i.e.* same column) labeled with a different letter indicate a significant difference ($p < 0.05$, Tukey's test)

Breakfast flakes derived from	Phytate content (g per 100 g dm)	Zn bioaccessibility (% of total Zn in flakes)	Total Zn content (mg kg^{-1} dm)	Molar phytate : Zn ratio (–)
Untreated wheat	0.87 ± 0.01^{a}	5.3 ± 0.4^{c}	24.6 ± 1.0^{a}	35.0
Steeped and sprouted wheat	0.64 ± 0.01^{b}	7.2 ± 0.2^{b}	32.0 ± 0.3^{b}	19.8
Hydrothermally processed wheat	0.44 ± 0.02^{c}	52.3 ± 0.3^{a}	18.2 ± 0.5^{c}	23.9

reduced by 91%. Similarly, hydrothermal processing of naked barley at 53–55 °C for 12 h in a citrate buffer (pH 4.8, unspecified cation and concentration) resulted in 54% phytate reduction.[49]

The bioaccessibility of Zn in the regular flakes was 5.3% of its total content (Table 1) and in line with that of untreated wheat reported by Huyskens *et al.*[22] Steeping (15 °C, 36 h) and sprouting (26 °C, 48 h) wheat reduced the phytate content by 26% (see above), resulting in mineral release and a 1.4-fold increase in Zn bioaccessibility (calculated as the ratio of that for flakes made from sprouted wheat compared to that for regular flakes). Earlier, Platel *et al.*[50] and Luo *et al.*[51] reported three- and two-fold increases in Zn bioaccessibility, respectively, when wheat was sprouted for 48 h at room temperature.

Huyskens *et al.*[22] found the bioaccessibility of Zn in hydrothermally processed wheat to be 50.4%. In the present study, it was 52.3% in breakfast flakes made from such processed wheat, and thus 9.9 times higher than that in flakes made from regular wheat. The high bioaccessibility can at least in part be attributed to the 48% phytate reduction caused by the hydrothermal processing (see above). Earlier, Lemmens *et al.*[13] observed a Zn bioaccessibility of only 35% despite an almost complete (*i.e.* 96%) phytate elimination in wheat processed at 50 °C and pH 3.8 for 24 h using 0.1 M sodium acetate buffer. In contrast, Huyskens *et al.*[22] reported a Zn bioaccessibility of 53%, even though the phytate was only reduced by 49% when wheat was processed at 60 °C and pH 4.0 for 24 h using 0.1 M sodium citrate buffer. Most likely, citrate enhances Zn solubility and bioaccessibility by forming soluble chelates.[52–54] Support for this view is that Ekholm *et al.*[55] reported a dose-dependent increase in Zn bioaccessibility (up to 80%) at citric acid concentrations (up to 30 mg g^{-1}) in oat bran and flakes. Additionally, Hemalatha *et al.*[52] found a 1.6-fold higher Zn bioaccessibility in cooked rice when adding 0.5% citric acid.

Moreover, solubility of Zn at intestinal pH, physical entrapment of the mineral within cells,[56] and binding to dietary fibers and polyphenols[57] may impact its bioaccessibility.

The Zn content in breakfast flakes derived from sprouted wheat was significantly higher than that of regular flakes (Table 1). This increase was at least mainly due to the uptake of Zn from the excess steeping water, which had a Zn concentration of 0.69 mg L^{-1}. Recently, Huyskens *et al.*[39] showed that the content of bioaccessible Zn in wheat did not increase as a result of steeping, indicating that the Zn originating from the steeping water was not bioaccessible (and therefore not bioavailable). Additionally, the Zn content of the breakfast flakes derived from sprouted wheat increased due to dry matter loss as result of respiration. In contrast, in the present work, hydrothermal processing of wheat in sodium citrate buffer resulted in reduced Zn content, most likely because of leaching of soluble Zn-citrate chelates into the incubation media. Additionally, Zn ions may also bind to compounds such as nicotinamide, deoxymugineic acid, or sulfate which are easily extracted from the wheat kernel,[22] resulting in lower Zn content. As a result, the test meals differed in total Zn content, highlighting the importance of assessing not only fractional Zn absorption but also the total Zn absorption (mg per portion) for each meal.

3.2 Study population

In this part of the study, it was investigated whether the 1.4-fold and 9.9-fold increases in Zn bioaccessibility observed in breakfast flakes made from sprouted and hydrothermally processed wheat, respectively (see 3.1), translated into increased Zn absorption in humans. The study flow diagram is presented in Fig. 1. Of the 47 participants who had expressed interest in participating in the study, 43 attended the screening visit, and 36 were enrolled in the study and randomized. All 36 participants completed the study, distributed over group I ($n = 24$) and group II ($n = 12$). The characteristics of the study population are shown in Table 2. The interindividual difference (stdev) in fractional Zn absorption from breakfast flakes derived from untreated wheat was 2.25%. This value was lower than the 7–9% found by Brnić *et al.*[37]

3.3 Zinc absorption

3.3.1 Impact of sprouting of wheat on Zn absorption. The average fractional Zn absorption from regular flakes was 5.1% (Table 3), which was in line with values reported earlier, *i.e.* 6.4%[40] and 8.9%[38] for whole wheat porridge. Although steeping (15 °C, 36 h) followed by sprouting (26 °C, 48 h) of wheat increased Zn bioaccessibility 1.4-fold (see 3.1), this improvement did not result in a corresponding increase in fractional Zn absorption (Table 3 and Fig. 2). This highlights the need for *in vivo* studies to validate *in vitro* findings and thus to deter-

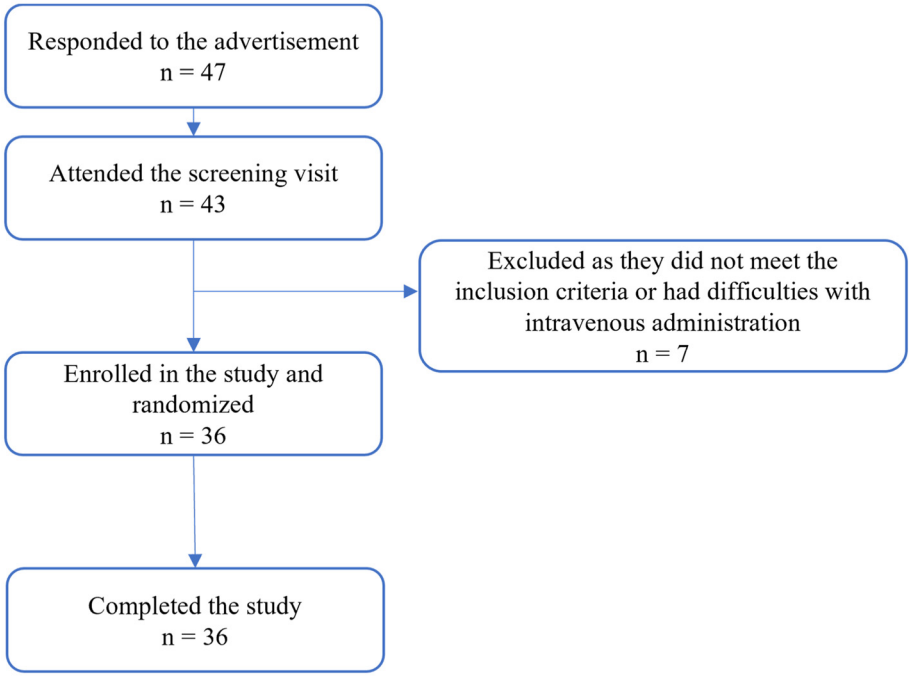

Fig. 1 Study flow diagram.

Table 2 Characteristics of the study population of group I (n = 24) and group II (n = 12). Results are presented as means ± standard deviations

Characteristic	Group I (steeped and sprouted *versus* untreated) n = 24	Group II (hydrothermally processed *versus* untreated) n = 12
Age (years)	27.1 ± 2.7	27.8 ± 2.9
Sex (male : female)	13 : 11	6 : 6
Length (cm)	173.4 ± 6.6	173.1 ± 8.7
Body weight (kg)	66.8 ± 7.9	64.4 ± 10.0
Body mass index (kg m^{-2})	22.2 ± 2.1	21.4 ± 2.2

Table 3 Weighted fractional zinc (Zn) absorption (%) and weighted total Zn absorption (mg per portion) from breakfast flakes derived from untreated wheat and from steeped (15 °C, 36 h) and sprouted (26 °C, 48 h) wheat. Data are presented for all participants (n = 24) and for a subset excluding those with fractional absorption values below the limit of quantification (n = 16). Mean values within a parameter (*i.e.* same row) labeled with a different letter indicate a significant difference (two-sided paired t-test, $p < 0.05$)

	Untreated wheat	Steeped and sprouted wheat	Two-sided paired t-test p-values
Fractional Zn absorption (%) (n = 24)	5.11 ± 2.25[a]	3.96 ± 3.42[a]	p = 0.27
Total Zn absorption (mg per portion) (n = 24)	0.13 ± 0.06[a]	0.13 ± 0.11[a]	p = 0.72
Fractional Zn absorption (%) (n = 16)	5.02 ± 1.87[a]	5.52 ± 3.19[a]	p = 0.53
Total Zn absorption (mg per portion) (n = 16)	0.12 ± 0.05[a]	0.18 ± 0.10[a]	p = 0.06

mine whether *in vitro* data can serve as a predictor for *in vivo* outcomes.

In contrast with the present study, Larsson *et al.*[58] reported a 1.6-fold increase in Zn absorption (n = 10) from porridge made from steeped (15 °C, 6.5 h) and sprouted (15 °C, 120 h) oats rather than from regular oats. Similarly, Fredlund *et al.*[59] observed a 1.5-fold increase in Zn absorption (n = 10) from flakes produced from steeped (13–15 °C, 34 h) and sprouted (15–17 °C, 6 days) barley instead of from barley. A likely cause of the discrepancy is the residual phytate : Zn molar ratio in the sprouted samples, which was lower in these studies than in the present study [*i.e.* 7.9 in the study of Larsson *et al.*[58] and 12.6 in the study of Fredlund *et al.*[59]]. Indeed, Nävert & Sandström[60] and Fredlund *et al.*[59] observed a strong inverse relation between phytate content and Zn absorption from whole meal bread (n = 42) and wheat-based porridges and breakfast flakes (n = 22), respectively. Furthermore, supplementing white wheat rolls (40 g) with up to 250 mg sodium phytate reduced Zn absorption (n = 40) from 22% to 6%. A significant decrease in absorption was observed when the phytate : Zn molar ratio was higher than 6.0.[61] In the present case, the phytate : Zn molar ratio in flakes made from whole meal of sprouted wheat was 19.8, whereas that in regular flakes was 35.0 (Table 1). Thus, an excess phytate was still

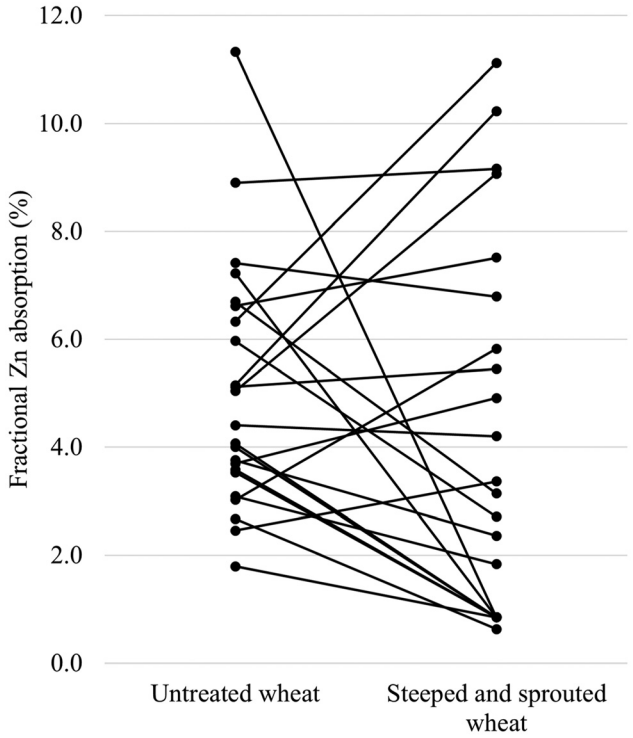

Fig. 2 Individual changes per participant (n = 24) for the fractional zinc (Zn) absorption (%) from breakfast flakes derived from untreated wheat and steeped (15 °C, 36 h) and sprouted (26 °C, 48 h) wheat. Each line represents a participant.

present in the flakes made from sprouted wheat, resulting in low Zn bioaccessibility and, consequently, limiting fractional Zn absorption. To meet the threshold proposed by Fredlund *et al.*,[61] a phytate breakdown of 78% would have been required to sufficiently enhance Zn bioaccessibility and bring about a substantial increase in fractional Zn absorption.

Another factor affecting fractional Zn absorption is the Zn content of the meal, which, as mentioned in 3.1, varied among the three types of flakes. Sandström *et al.*[62] observed that adding 3.3 mg Zn in the form of Zn chloride to composite meals of chicken (originally containing 1.3 mg Zn) and beef (originally containing 4.6 mg Zn) resulted in a 1.3- and 1.5-fold reduction in fractional Zn absorption, respectively. This may partly be due to the homeostatic regulation of Zn in non-deficient individuals. Zinc homeostasis is maintained *via* absorption of dietary Zn and secretion and excretion of endogenous Zn. Fractional Zn absorption is inversely related to dietary bioaccessible Zn intake. Therefore, the additional bioaccessible Zn resulting from sprouting is unlikely to be absorbed, which results in decreases in fractional Zn absorption.[63]

In addition, heat treatment during the production may lead to formation of Zn complexes that cannot be absorbed.[64] However, Fairweather-Tait *et al.*[65] observed that the extrusion of whole wheat flour for flake production did not affect *in vivo* (n = 11) Zn absorption. Furthermore, sprouting releases reducing sugars and amino groups,[56] which upon heating form Maillard products that may chelate Zn, thereby inhibiting its absorption.[64,66]

Fractional Zn absorption for 8 of the 24 participants was below the limit of quantification (*i.e.* 0.85%) of the method used. Assigning the limit of quantification to these cases reduced the variability and introduced bias into the results. To test this potential effect, these participants were either or not excluded from the analysis. When they were excluded, fractional Zn absorption from flakes derived from sprouted wheat increased but did again not differ from that of regular flakes. Nevertheless, total Zn absorption from breakfast flakes made from sprouted wheat was higher (p = 0.06) than that from regular flakes, corresponding to a 1.4-fold increase in total Zn absorption.

This increase is partly attributed to dry matter loss during sprouting, which led to higher Zn content in flakes made from sprouted wheat compared to regular flakes. The Zn in the flakes originating from the steeping water did not contribute to total Zn absorption, as it was not bioaccessible and therefore not bioavailable (see 3.1). It is of further note that the Fe content of the wheat flakes increased during the flake production process as a result of contact of the breakfast pellets with the equipment used (SI, Table S5). Although Fe may negatively affect Zn absorption due to competition for specific transport proteins at the intestinal membrane,[67] such negative effects have only been observed at much higher Fe : Zn molar ratios than those in the present test meals, and primarily with aqueous solutions rather than with food matrices.[68] Indeed, Donangelo *et al.*[69] observed that Fe supplementation in women (n = 12) with marginal iron status did not affect Zn absorption from cooked kidney beans.

In conclusion, sprouting of wheat did not result in increased fractional or total Zn absorption values (n = 24) from breakfast flakes derived thereof. This is primarily attributed to the high residual phytate : Zn ratio of 19.8 in the flakes, which is well above the threshold of 6.0 noted by Fredlund *et al.*[61] Therefore, a more extensive breakdown of phytate would be required to achieve an increase in Zn absorption.

Table 4 Weighted fractional zinc (Zn) absorption (%) and weighted total Zn absorption (mg per portion) from breakfast flakes derived from untreated wheat and from hydrothermally processed (60 °C, pH 4.0, 24 h) wheat using a 0.1 M sodium citrate buffer. Mean values within a parameter (*i.e.* same row) labeled with a different letter indicate a significant difference (two-sided paired t-test, $p < 0.05$)

	Untreated wheat	Hydrothermally processed wheat	Two-sided paired t-test p-values
Fractional Zn absorption (%) (n = 12)	6.38 ± 4.41[a]	7.04 ± 3.98[a]	p = 0.11
Total Zn absorption (mg per portion) (n = 12)	0.21 ± 0.19[a]	0.16 ± 0.10[a]	p = 0.64

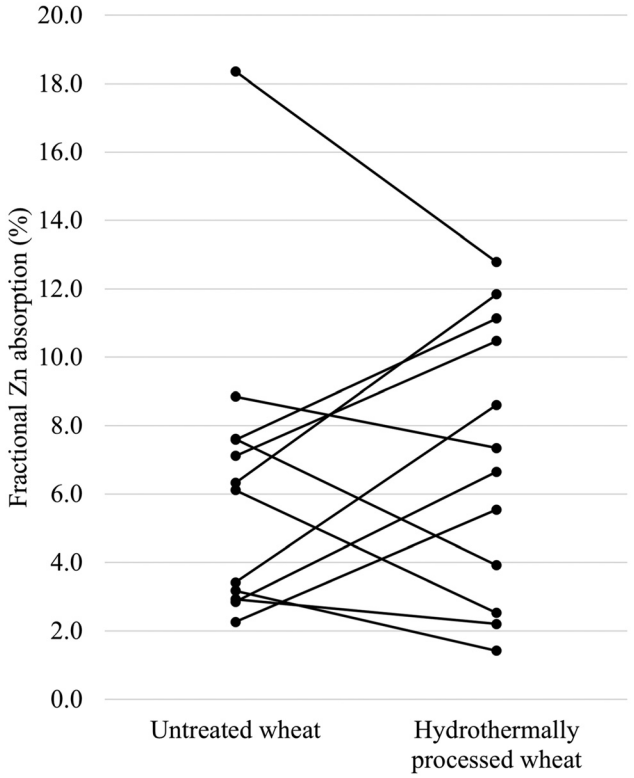

Fig. 3 Individual changes per participant (n = 12) for the fractional zinc (Zn) absorption (%) from breakfast flakes derived from untreated wheat and from hydrothermally processed (60 °C, pH 4.0, 24 h) wheat using a 0.1 M sodium citrate buffer. Each line represents a participant.

3.3.2 Impact of hydrothermal processing of wheat on Zn absorption. The average fractional Zn absorption from flakes derived from hydrothermally processed wheat amounted to 7.0% (Table 4), which was higher (p = 0.11) than the fractional absorption from regular flakes (6.4%). The 9.9-fold increase in Zn bioaccessibility for flakes made from hydrothermally processed wheat (see 3.1) was translated into a modest 1.1-fold increase in fractional Zn absorption (Table 4 and Fig. 3). Similarly, Fredlund *et al.*[59] reported a 2.3-fold increase in Zn absorption (n = 12) from porridge of hydrothermally processed (48–50 °C, 4 h, pH 2.5, lactic acid solution) barley, which had a phytate : Zn molar ratio 1.6, compared to 16.5 in porridge from untreated barley. In the present study, the phytate : Zn molar ratio decreased from 35.0 in regular flakes to 23.9 in flakes of hydrothermally processed wheat. This high residual phytate : Zn molar ratio is an explanation for the limited increase in fractional Zn absorption. To meet the threshold proposed by Fredlund *et al.*[61] (see 3.3.1), approximately 87% of the phytate would need to be degraded, whereas only a 48% phytate reduction was achieved in the present study.

Additionally, as mentioned in 3.1, citrate forms soluble chelates with Zn, thereby increasing its bioaccessibility.[68] While Huyskens *et al.*[22] reported a 12-fold increase in fractional Zn absorption in Caco-2 cells as a result of hydrothermal processing of wheat under conditions identical to those used in the present study, the same process resulted in only a modest increase in *in vivo* fractional Zn absorption. Thus, it is important to investigate whether increases observed *in vitro* also translate into higher *in vivo* values. Pabón[70] observed a 10–20% increase in Zn absorption in rats when 5 mM citrate was added to meals containing various sources of casein and whey proteins. In contrast, Lönnerdal *et al.*[71] concluded from an *in vivo* study (n = 6) that potassium citrate did not improve Zn absorption from soy-based infant formula. A possible explanation for the modest increase in fractional Zn absorption is that a substantial fraction of the bioaccessible Zn, including Zn-citrate chelates, is *in vivo* non-labile and therefore does not readily release Zn ions for uptake by the enterocytes.

Although Zn homeostasis limits increases in fractional Zn absorption with higher bioaccessible Zn intake (see section 3.3.1), a small increase in fractional Zn absorption was observed in this study. At the intestinal site, there is competition for absorption from dietary Zn and secreted Zn (*e.g.* pancreatic and biliary secretions). These results suggest that dietary Zn is more readily absorbed than secreted Zn. It has been shown that at high levels of bioaccessible Zn, such as resulting from hydrothermal processing of wheat, Zn homeostasis may be regulated through increased Zn excretion rather than reduced absorption.[63]

Unfortunately, the flakes derived from hydrothermally processed wheat contained less Zn than the regular flakes, likely due to leaching out of the mineral in the incubation media (see 3.1). As a result, the total Zn absorption from flakes derived from hydrothermally processed wheat (0.16 mg per portion) did not differ from the total Zn absorption from regular flakes (0.21 mg per portion).

To conclude, prior hydrothermal processing of wheat reduced the phytate : Zn molar ratio in flakes from 35.0 to 23.9. However, this value is well above the threshold of 6.0 listed by Fredlund *et al.*,[61] which may well be the cause of the limited increase in fractional Zn absorption. Be as it may, more profound phytate breakdown is needed in order to enhance Zn absorption extensively.

The small or insignificant effects of both wheat processing treatments on Zn absorption may also be related to the selected method to measure Zn absorption from a food product. The single-meal method is commonly used in dietary Zn uptake studies due to its convenience.[19,21,72] However, it does not reflect long-term Zn absorption from sustained consumption of that food product. In this study, both types of breakfast flakes were consumed only once and with only 24 h apart, assuming no influence from Zn and/or phytate content in meals consumed before, between or after the breakfast flakes. Standard meals, selected for their low phytate and Zn content, were provided for lunch and dinner on the days breakfast flakes were consumed. However, no standard meals were provided the day prior to the study or the days after the study. Although participants were asked to fast overnight prior to consuming the breakfast flakes and to delay their lunch for at least 3 h after consuming the flakes, prior meals and meals after the second breakfast may have influenced Zn absorption

in the intestinal tract. An alternative approach [as used by Rosado *et al.*[72]] would have been to have the participants also exclusively consume breakfast flakes during lunch and dinner.

4. Conclusions

The aim of this study was to evaluate the effect of sprouting and hydrothermal processing of wheat on Zn bioaccessibility and its absorption in the human body. In essence, a human intervention study was carried out using a dual isotope technique, where breakfast flakes were extrinsically labeled with enriched stable Zn isotopes. Twelve healthy participants consumed regular flakes as well as flakes from sprouted wheat. In a separate group, 24 healthy participants consumed regular wheat flakes alongside flakes produced from hydrothermally processed wheat.

Despite the increased Zn bioaccessibility observed in breakfast flakes made from steeped (15 °C, 36 h) and sprouted (26 °C, 48 h) wheat, these flakes did not result in better fractional or total Zn absorption (n = 24) than noted for regular breakfast flakes. Hydrothermal processing (60 °C, pH 4.0, 24 h) of wheat in 0.1 M sodium citrate buffer largely increased Zn bioaccessibility through substantial phytate reduction and formation of soluble Zn-citrate chelates. However, this led to a modest increase in fractional Zn absorption only (n = 12). In the end, total Zn absorption did not differ significantly between flakes, as those from hydrothermally processed wheat contained less Zn.

Both sprouting of wheat and hydrothermal processing of wheat resulted in phytate : Zn molar ratios well above the threshold of 6.0 proposed by Fredlund *et al.*,[61] likely explaining the modest to absent increases in fractional Zn absorption. These findings illustrate that a greater breakdown of phytate is necessary to substantially enhance Zn absorption.

Thus, claims regarding increased Zn bioaccessibility due to phytate reduction in food products should be interpreted with caution, as increased bioaccessibility does not necessarily lead to better Zn absorption from a single consumption of that product. Human intervention studies are needed to confirm actual improvements in Zn absorption *in vivo*.

Author contributions

Marie Huyskens: conceptualization, data curation, validation, investigation, formal analysis, writing – original draft, writing – review & editing. Elien Lemmens: conceptualization, writing – review & editing. Lana Abou-Zeid: data curation, writing – review & editing. Kasper Hobin: data curation, writing – review & editing. Lukas M. Balsiger: investigation, writing – review & editing. Frank Vanhaecke: writing – review & editing, resources. Kristin Verbeke: funding acquisition, writing – review & editing. Erik Smolders: conceptualization, writing – review & editing, supervision, resources. Jan A. Delcour: conceptualization, funding acquisition, writing – review & editing, supervision, resources.

Conflicts of interest

The authors declare that they have no competing interests. This research was conducted in the framework of the W. K. Kellogg Chair in Cereal Science at the KU Leuven (chair holders J. A. Delcour and K. Verbeke).

List of abbreviations

d_{iv}	Dose of intravenously administered isotopic tracer
dm	Dry matter
d_{oral}	Dose of orally administered isotopic tracer
ICP-MS	Inductively coupled plasma-mass spectrometry
MC-ICP-MS	Multicollector inductively coupled plasma-mass spectrometry
NIST	National Institute of Standards and Technology
n_{oral}	Enrichment of orally administered isotopic tracer
n_{iv}	Enrichment of intravenously administered isotopic tracer
rpm	Rotations per minute

Data availability

The data is available from RDR KU Leuven data portal at https://doi.org/10.48804/JLKZOR.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d5fo03650j.

Acknowledgements

The authors thank W.K. Kellogg Chair in Cereal Science at KU Leuven for funding this work. Frank Vanhaecke acknowledges FWO-Vlaanderen for providing the funding for the acquisition of MC-ICP-MS instrumentation (ZW15-02-G0H6216 N).

During the preparation of this work the authors used Microsoft 365 Copilot in order to improve readability and language of the work. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

References

1 FAO, https://www.fao.org/worldfoodsituation/csdb/, (accessed February 7, 2025).
2 FAS, https://www.fas.usda.gov/data/production/commodity/0410000, (accessed February 7, 2025).

3 J. A. Delcour and R. C. Hoseney, *Principles of Cereal Science and Technology*, AACC International Inc., St. Paul, MN, USA, 3rd edn, 2010.
4 J. Liu, L. L. Yu and Y. Wu, Bioactive components and health beneficial properties of whole wheat foods, *J. Agric. Food Chem.*, 2020, **68**, 12904–12915.
5 F. Brouns, Y. Hemery, R. Price and N. M. Anson, Wheat aleurone: Separation, composition, health aspects, and potential food use, *Crit. Rev. Food Sci. Nutr.*, 2012, **52**, 553–568.
6 V. Piironen, A. M. Lampi, P. Ekholm, M. Salmenkallio-Marttila and K. H. Liukkonen, in *Wheat - Chemistry and Technology*, ed. K. Khan and P. R. Shewry, AACC International Inc, St. Paul, MN, USA, 4th edn, 2009, 7, 179–222.
7 B. Bates, A. Lennox, A. Prentice, C. Bates, P. Page, S. Nicholson and G. Swan, *National diet and nutrition survey results from years 1,2,3 and 4 (combined) of the rolling programme (2008/2009–2011/2012)*, Public Health England and The Food Standards Agency, UK, 2017.
8 A. Whatham, H. Bartlett, F. Eperjesi, C. Blumenthal, J. Allen, C. Suttle and K. Gaskin, Vitamin and mineral deficiencies in the developed world and their effect on the eye and vision, *Ophthalmic Physiol. Opt.*, 2008, **28**, 1–12.
9 K. R. Wessells and K. H. Brown, Estimating the global prevalence of zinc deficiency: Results based on zinc availability in national food supplies and the prevalence of stunting, *PLoS One*, 2012, **7**, e50568.
10 EFSA NDA Panel, Scientific opinion on dietary reference values for zinc, *EFSA J.*, 2014, **12**, 3844.
11 J. M. Carbonell-Capella, M. Buniowska, F. J. Barba, M. J. Esteve and A. Frígola, Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A review, *Compr. Rev. Food Sci. Food Saf.*, 2014, **13**, 155–171.
12 C. M. Galanakis and S. R. Drago, in *Nutraceutical and Functional Food Components*, ed. C. M. Galanakis, Academic Press, Cambridge, MA, USA, 2nd edn, 2022, vol. 1, pp. 1–18.
13 E. Lemmens, N. De Brier, K. M. Spiers, C. Ryan, J. Garrevoet, G. Falkenberg, P. Goos, E. Smolders and J. A. Delcour, The impact of steeping, germination and hydrothermal processing of wheat (Triticum aestivum L.) grains on phytate hydrolysis and the distribution, speciation and bio-accessibility of iron and zinc elements, *Food Chem.*, 2018, **264**, 367–376.
14 M. Sultana, T. Hasan and N. Shaheen, Molar ratios of dietary phytate to minerals and iron status of female residential students in University of Dhaka, Bangladesh, *Nutr. Health*, 2021, **27**, 405–412.
15 Y. Y. Zhang, R. Stockmann, K. Ng and S. Ajlouni, Revisiting phytate-element interactions: Implications for iron, zinc and calcium bioavailability, with emphasis on legumes, *Crit. Rev. Food Sci. Nutr.*, 2022, **62**, 1696–1712.
16 X. G. Lei and J. M. Porres, Phytase enzymology, applications, and biotechnology, *Biotechnol. Lett.*, 2003, **25**, 1787–1794.
17 T. H. Iqbal and B. T. Cooper, Phytase activity in the human and rat small intestine, *Gut*, 1994, **35**, 1233–1236.
18 A. S. Sandberg and H. Andersson, Effect of dietary phytase on the digestion of phytate in the stomach and small intestine of humans, *J. Nutr.*, 1988, **118**, 469–473.
19 M. Brnić, R. Wegmüller, C. Zeder, G. Senti and R. F. Hurrell, Influence of phytase, EDTA, and polyphenols on zinc absorption in adults from porridges fortified with zinc sulfate or zinc oxide, *J. Nutr.*, 2014, **144**, 1467–1473.
20 I. Egli, L. Davidsson, C. Zeder, T. Walczyk and R. F. Hurrell, Dephytinization of a complementary food based on wheat and soy increases zinc, but not copper, apparent absorption in adults, *J. Nutr.*, 2004, **134**, 1077–1080.
21 M. K. Hambidge, J. W. Huffer, V. Raboy, G. K. Grunwald, J. L. Westcott, L. Sian, L. V. Miller, J. A. Dorsch and N. F. Krebs, Zinc absorption from low-phytate hybrids of maize and their wild-type isohybrids, *Am. J. Clin. Nutr.*, 2004, **79**, 1053–1062.
22 M. Huyskens, E. Lemmens, C. Grootaert, J. Van Camp, K. Verbeke, P. Goos, E. Smolders and J. A. Delcour, Acidic hydrothermal processing of wheat using citrate buffer largely enhances iron and zinc bioaccessibility and bioavailability to Caco-2 cells, *Food Chem.*, 2025, **467**, 142340.
23 J. R. Turnlund, J. C. King, W. R. Keyes, B. Gong and M. C. Michel, A stable isotope study of zinc absorption in young men: Effects of phytate and a-cellulose, *Am. J. Clin. Nutr.*, 1984, **40**, 1071–1077.
24 I. El Houssni, A. Zahidi, K. Khedid and R. Hassikou, Review of processes for improving the bioaccessibility of minerals by reducing the harmful effect of phytic acid in wheat, *Food Chem. Adv.*, 2024, **4**, 100568.
25 J. D. Bewley, Seed germination and dormancy, *Plant Cell*, 1997, **9**, 1055–1056.
26 F. A. Guzmán-Ortiz, J. Castro-Rosas, C. A. Gómez-Aldapa, R. Mora-Escobedo, A. Rojas-León, M. L. Rodríguez-Marín, R. N. Falfán-Cortés and A. D. Román-Gutiérrez, Enzyme activity during germination of different cereals: A review, *Food Rev. Int.*, 2019, **35**, 177–200.
27 E. Lemmens, N. De Brier, P. Goos, E. Smolders and J. A. Delcour, Steeping and germination of wheat (Triticum aestivum L.). I. Unlocking the impact of phytate and cell wall hydrolysis on bio-accessibility of iron and zinc elements, *J. Cereal Sci.*, 2019, **90**, 102847.
28 N. F. Krebs, L. V. Miller, V. L. Naake, S. Lei, J. E. Westcott, P. V. Fennessey and M. K. Hambidge, The use of stable isotope techniques to assess zinc metabolism, *J. Nutr. Biochem.*, 1995, **6**, 292–301.
29 International Atomic Energy Agency, *Assessment of zinc metabolism in humans using stable zinc isotope techniques*, IAEA Human Health series, Vienna, Austria, 2018, vol. 35.
30 J. K. Friel, V. L. Naake, L. V. Miller, P. V. Fennessey and M. K. Hambidge, The analysis of stable isotopes in urine to determine the fractional absorption of zinc, *Am. J. Clin. Nutr.*, 1992, **55**, 473–477.
31 L. Yang, X. Yang, J. Piao, Y. Tian, P. Li, Y. Wang and J. Wang, Studies on zinc bioavailability from a representa-

tive diet in Chinese urban women of childbearing age using a double label stable isotope technique, *J. Trace Elem. Med. Biol.*, 2005, **19**, 159–164.

32 N. F. Krebs and M. K. Hambidge, Zinc metabolism and homeostasis: The application of tracer techniques to human zinc physiology, *BioMetals*, 2001, **14**, 397–412.

33 R. Wegmüller, F. Tay, C. Zeder, M. Brnić and R. F. Hurrell, Zinc absorption by young adults from supplemental zinc citrate is comparable with that from zinc gluconate and higher than from zinc oxide, *J. Nutr.*, 2014, **144**, 132–136.

34 C. M. Donangelo, L. R. Woodhouse, S. M. King, G. Toffolo, D. M. Shames, F. E. Viteri, Z. Cheng, R. M. Welch and J. C. King, Iron and zinc absorption from two bean (Phaseolus vulgaris L.) genotypes in young women, *J. Agric. Food Chem.*, 2003, **51**, 5137–5143.

35 X. Y. Sheng, M. K. Hambidge, L. V. Miller, J. E. Westcott, S. Lei and N. F. Krebs, Measurement of zinc absorption from meals: Comparison of extrinsic zinc labeling and independent measurements of dietary zinc absorption, *Int. J. Vitam. Nutr. Res.*, 2009, **79**, 230–237.

36 L. Davidsson, A. Cederblad, E. Hagebø, B. Lönnerdal and B. Sandström, Intrinsic and extrinsic labeling for studies of manganese absorption in humans, *J. Nutr.*, 1988, **118**, 1517–1521.

37 M. Brnić, R. Wegmüller, A. Melse-Boonstra, T. J. Stomph, C. Zeder, F. M. Tay and R. F. Hurrell, Zinc absorption by adults is similar from intrinsically labeled zinc-biofortified rice and from rice fortified with labeled zinc sulfate, *J. Nutr.*, 2016, **146**, 76–80.

38 C. Signorell, M. B. Zimmermann, I. Cakmak, R. Wegmüller, C. Zeder, R. Hurrell, S. B. Aciksoz, F. Tay, E. Frossard and D. Moretti, Zinc absorption from agronomically biofortified wheat is similar to post-harvest fortified wheat and is a substantial source of bioavailable zinc in humans, *J. Nutr.*, 2019, **149**, 840–846.

39 M. Huyskens, E. Lemmens, C. Grootaert, J. Van Camp, K. Verbeke, J. A. Delcour and E. Smolders, The impact of wheat sprouting on iron and zinc bioaccessibility and bioavailability to Caco-2 cells, *Food Chem.*, 2025, **495**, 146332.

40 D. L. De Romaña, B. Lönnerdal and K. H. Brown, Absorption of zinc from wheat products fortified with iron and either zinc sulfate or zinc oxide, *Am. J. Clin. Nutr.*, 2003, **78**, 279–283.

41 L. Van Heghe, E. Engström, I. Rodushkin, C. Cloquet and F. Vanhaecke, Isotopic analysis of the metabolically relevant transition metals Cu, Fe and Zn in human blood from vegetarians and omnivores using multi-collector ICP-mass spectrometry, *J. Anal. At. Spectrom.*, 2012, **27**, 1327–1334.

42 D. C. Baxter, I. Rodushkin, E. Engström and D. Malinovsky, Revised exponential model for mass bias correction using an internal standard for isotope abundance ratio measurements by multi-collector inductively coupled plasma mass spectrometry, *J. Anal. At. Spectrom.*, 2006, **21**, 427–430.

43 R. E. T. Moore, F. Larner, B. J. Coles and M. Rehkämper, High precision zinc stable isotope measurement of certified biological reference materials using the double spike technique and multiple collector-ICP-MS, *Anal. Bioanal. Chem.*, 2017, **409**, 2941–2950.

44 R. E. T. Moore, M. Rehkämper, W. Maret and F. Larner, Assessment of coupled Zn concentration and natural stable isotope analyses of urine as a novel probe of Zn status, *Metallomics*, 2019, **11**, 1506–1517.

45 P. R. Flanagan, J. Cluett, M. J. Chamberlain and L. S. Valberg, Dual-isotope method for determination of human zinc absorption: The use of a test meal of turkey meat, *J. Nutr.*, 1985, **115**, 111–122.

46 D. M. Dias, N. M. B. Costa, M. R. Nutti, E. Tako and H. S. D. Martino, Advantages and limitations of in vitro and in vivo methods of iron and zinc bioavailability evaluation in the assessment of biofortification program effectiveness, *Crit. Rev. Food Sci. Nutr.*, 2018, **58**, 2136–2146.

47 N. R. Reddy, S. K. Sathe and D. K. Salunkhe, Phytates in legumes and cereals, *Adv. Food Res.*, 1982, **28**, 1–92.

48 M. Bartnik and I. Szafrańska, Changes in phytate content and phytase activity during the germination of some cereals, *J. Cereal Sci.*, 1987, **5**, 23–28.

49 K. Fredlund, N. G. Asp, M. Larsson, I. Marklinder and A. S. Sandberg, Phytate reduction in whole grains of wheat, rye, barley and oats after hydrothermal treatment, *J. Cereal Sci.*, 1997, **25**, 83–91.

50 K. Platel, S. W. Eipeson and K. Srinivasan, Bioaccessible mineral content of malted finger millet (Eleusine coracana), wheat (Triticum aestivum), and barley (Hordeum vulgare), *J. Agric. Food Chem.*, 2010, **58**, 8100–8103.

51 Y. W. Luo, W. H. Xie, X. X. Jin, Q. Wang and Y. J. He, Effects of germination on iron, zinc, calcium, manganese, and copper availability from cereals and legumes, *CyTA – J. Food*, 2014, **12**, 22–26.

52 S. Hemalatha, K. Platel and K. Srinivasan, Influence of food acidulants on bioaccessibility of zinc and iron from selected food grains, *Mol. Nutr. Food Res.*, 2005, **49**, 950–956.

53 S. Z. H. Shah, M. Fatima, M. Afzal and M. Bilal, Interactive effect of citric acid, phytase and chelated mineral on growth performance, nutrient digestibility and whole-body composition of Labeo rohita fingerlings, *Aquacult. Res.*, 2020, **52**, 842–858.

54 N. Tang and L. H. Skibsted, Zinc bioavailability from phytate-rich foods and zinc supplements. Modeling the effects of food components with oxygen, nitrogen, and sulfur donor ligands, *J. Agric. Food Chem.*, 2017, **65**, 8727–8743.

55 P. Ekholm, L. Virkki, M. Ylinen, L. Johansson and P. Varo, Effects of natural chelating agents on the solubility of some physiologically important mineral elements in oat bran and oat flakes, *Cereal Chem.*, 2000, **77**, 562–566.

56 E. Lemmens, L. J. Deleu, N. De Brier, E. Smolders and J. A. Delcour, Mineral bio-accessibility and intrinsic saccharides in breakfast flakes manufactured from sprouted wheat, *LWT – Food Sci. Technol.*, 2021, **143**, 111079.

57 S. Rousseau, C. Kyomugasho, M. Celus, M. E. G. Hendrickx and T. Grauwet, Barriers impairing mineral bioaccessibility

and bioavailability in plant-based foods and the perspectives for food processing, *Crit. Rev. Food Sci. Nutr.*, 2020, **60**, 826–843.

58 M. Larsson, L. Rossander-Hulthén, B. Sandström and A. S. Sandberg, Improved zinc and iron absorption from breakfast meals containing malted oats with reduced phytate content, *Br. J. Nutr.*, 1996, **76**, 677–688.

59 K. Fredlund, E. L. Bergman, L. Rossander-Hulthén, M. Isaksson, A. Almgren and A. S. Sandberg, Hydrothermal treatment and malting of barley improved zinc absorption but not calcium absorption in humans, *Eur. J. Clin. Nutr.*, 2003, **57**, 1507–1513.

60 B. Nävert and B. Sandström, Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man, *Br. J. Nutr.*, 1985, **53**, 47–53.

61 K. Fredlund, M. Isaksson, L. Rossander-Hulthén, A. Almgren and A. S. Sandberg, Absorption of zinc and retention of calcium: Dose-dependent inhibition by phytate, *J. Trace Elem. Med. Biol.*, 2006, **20**, 49–57.

62 B. Sandström and A. Cederblad, Zinc absorption from composite meals II. Influence of the main protein source, *Am. J. Clin. Nutr.*, 1980, **33**, 1778–1783.

63 N. F. Krebs, Overview of zinc absorption and excretion in the human gastrointestinal tract, *J. Nutr.*, 2000, **130**, 1374S–1377S.

64 M. R. La Frano, F. F. de Moura, E. Boy, B. Lönnerdal and B. J. Burri, Bioavailability of iron, zinc, and provitamin A carotenoids in biofortified staple crops, *Nutr. Rev.*, 2014, **72**, 289–307.

65 S. J. Fairweather-Tait, D. E. Portwood, L. L. Symss, J. Eagles and M. J. Minski, Iron and zinc absorption in human subjects from a mixed meal of extruded and nonextruded wheat bran and fIour, *Am. J. Clin. Nutr.*, 1989, **49**, 151–155.

66 S. Perales, R. Barberá, M. J. Lagarda and R. Farré, Bioavailability of zinc from infant foods by in vitro methods (solubility, dialyzability and uptake and transport by Caco-2 cells), *J. Agric. Food Chem.*, 2006, **86**, 971–978.

67 P. Kondaiah, P. S. Yaduvanshi, P. A. Sharp and R. Pullakhandam, Iron and zinc homeostasis and interactions: Does enteric zinc excretion cross-talk with intestinal iron absorption?, *Nutrients*, 2019, **11**, 1885.

68 B. Lönnerdal, Dietary factors influencing zinc absorption, *J. Nutr.*, 2000, **130**, 1378S–1383S.

69 C. M. Donangelo, L. R. Woodhouse, S. D. Mertz, F. E. Viteri and J. C. King, Supplemental zinc lowers measures of iron status in young women with low iron reserves, *J. Nutr.*, 2002, **132**, 1860–1864.

70 M. L. Pabón and B. Lönnerdal, Effect of citrate on zinc bioavailability from milk, milk fractions and infant formulas, *Nutr. Res.*, 1993, **13**, 103–111.

71 B. Lönnerdal, A. Cederblad, L. Davidsson and B. Sandstrom, The effect of individual components of soy formula and cows' milk formula on zinc bioavailability, *Am. J. Clin. Nutr.*, 1984, **40**, 1064–1070.

72 J. L. Rosado, M. K. Hambidge, L. V. Miller, O. P. Garcia, J. Westcott, K. Gonzalez, J. Conde, C. Hotz, W. Pfeiffer, I. Ortiz-Monasterio and N. F. Krebs, The quantity of zinc absorbed from wheat in adult women is enhanced by biofortification, *J. Nutr.*, 2009, **139**, 1920–1925.