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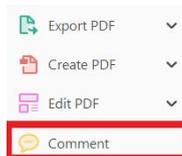
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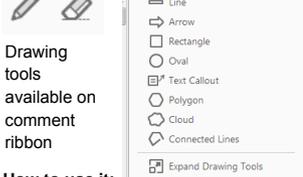
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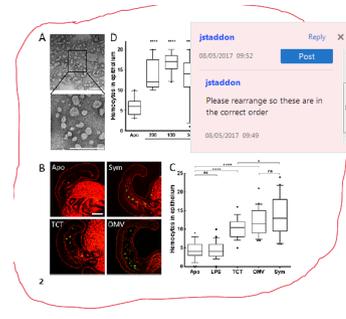


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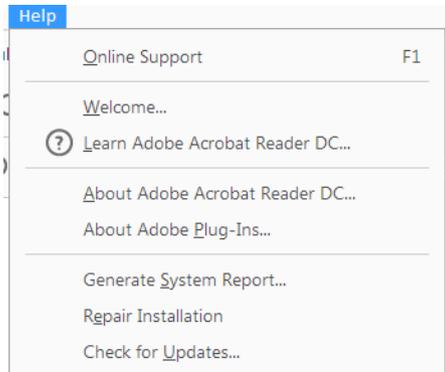
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Improving wheat as a source of iron and zinc for global nutrition

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Abstract

Wheat is the staple food crop in temperate countries and increasingly consumed in developing countries, displacing traditional foods. However, wheat products are typically low in bioavailable iron and zinc, contributing to deficiencies in these micronutrients in countries where wheat is consumed as a staple food. Two factors contribute to the low contents of bioavailable iron and zinc in wheat: the low concentrations of these minerals in white flour, which is most widely consumed, and their presence as phytates in mineral-rich bran fractions. Although high zinc types of wheat have been developed by conventional plant breeding (biofortification), this approach has failed for iron. However, studies in wheat and other cereals have shown that transgenic (also known as genetically modified; GM) strategies can be used to increase the contents of iron and zinc in white flour, by converting the starchy endosperm tissue into a 'sink' for minerals. Although such strategies currently have low acceptability, greater understanding of the mechanisms which control the transport and deposition of iron and zinc in the developing grain should allow similar effects to be achieved by exploiting naturally induced genetic variation. When combined with conventional biofortification and innovative processing, this approach should provide increased mineral bioavailability in a range of wheat products, from white flour to wholemeal.

Keywords: bioavailability, biofortification, iron, phytic acid, wheat, zinc

Introduction

It is difficult to overemphasise the global importance of deficiencies of mineral micronutrients, principally of iron and zinc, in human diets. It has been estimated

that globally 43% of children and 29% of women of reproductive age have anaemia, and about half of these cases result from iron deficiency (WHO 2015). Zinc deficiency is associated with stunted growth in children under the age of 5 years and reported to affect approximately 155 million children globally (WHO 2013). In the UK, 25% of the population aged 19–64 years experience zinc deficiency, while 20% of women aged 11–64 years (especially pregnant women)

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1 have a high risk of iron deficiency (Heath &
2 Fairweather-Tait 2002). Cereals such as wheat, rice
3 and maize provide 30–35% of energy intake in the
4 UK (Bates *et al.* 2014) and up to 60% of the daily
5 calories in developing countries (Ritchie & Roser
6 2018), but conventional processing of the grains
7 removes most of the micronutrients.

8 Many countries have mandatory fortification for
9 iron and selected vitamins in flours from wheat, maize
10 and rice (www.ffinetwork.org). Fortification of cereal
11 flour with zinc is also practiced in several countries,
12 but largely on a voluntary basis (Brown *et al.* 2010).
13 All white wheat flour milled in the UK is fortified in
14 accordance with the Flour and Bread Regulations
15 1998 ([http://www.legislation.gov.uk/ukxi/1998/141/c](http://www.legislation.gov.uk/ukxi/1998/141/contents/made)
16 [ontents/made](http://www.legislation.gov.uk/ukxi/1998/141/contents/made)) with inorganic forms of iron at levels of
17 16.5 mg/kg (equivalent to levels in ~~wheat flour at an~~
18 ~~80% extraction rate~~), which then enters the food
19 chain through bread, pasta, noodles, cakes, biscuits
20 and a range of other products. In other parts of the
21 world where wheat is a staple crop, such as north
22 India and Pakistan, fortification is difficult to imple-
23 ment because milling is carried out domestically at a
24 small scale as well as in large-scale industrial mills.
25 Hence, more innovative strategies are required to
26 ensure sustainable micronutrient levels in those regions
27 that need it most.

28 The provision of adequate minerals from bread and
29 other cereal products is determined by their total
30 amount in the grain and by their bioavailability.
31 Although the latter can be increased, to a limited
32 extent, by post-harvest processing such as micro-
33 milling and fermentation, modern breeding approaches
34 may be the only way to achieve the profound
35 increases in the amounts of bioavailable iron and zinc
36 that are required to meet the metabolic demands of
37 the global population, by altering the localisation and
38 form of the minerals in the grain.

41 Location of iron and zinc in wheat grains

42 The wheat grain is a single-seeded fruit, called a cary-
43opsis. It contains a small embryo, which forms in the
44 new plant on germination, and a large storage tissue
45 (the endosperm), which comprises mainly starch (a
46 source of energy) and protein. These tissues are sur-
47 rounded by protective layers derived from the seed coat
48 (testa) and fruit coat (pericarp). Iron and zinc, together
49 with other minerals, are concentrated in the embryo
50 and in the outer layer of endosperm cells, called the
51 aleurone. These distributions can be clearly seen in sec-
52 tions of a wheat grain using simple staining methods

(Fig. 1a), but higher resolution is obtained by modern
imaging systems such as Synchrotron X-ray fluores-
cence (Fig. 1b) and NanoSIMS (secondary ion mass
spectroscopy) (Fig. 1c; Moore *et al.* 2012; Neal *et al.*
2013). The uneven distribution of the two minerals
makes sense from a plant biology perspective: upon ger-
mination of the seed, the embryo will grow rapidly into
a young seedling for which it requires enzymes that are
dependent on iron, zinc and other cofactors (Bastow
et al. 2018). The growth is sustained by energy and
amino acids derived from the storage reserves (starch
and protein) in the central starchy endosperm. How-
ever, because the starchy endosperm cells die during the
later stages of grain maturation (Young & Gallie
2000), the starch and protein are mobilised as sugars
and amino acids after lytic digestion by enzymes
secreted from the aleurone layer and embryo. Interest-
ingly, the two minerals differ slightly in distribution
between the two tissues, with iron being more concen-
trated in the aleurone and zinc in the embryo (as shown
in Fig. 1b). The biological significance of these differ-
ences in the locations of the two minerals is not known.

Traditional milling of wheat by grinding between
stones produces wholemeal flour in which all parts of
the grain are mixed and can be only partially separ-
ated by sieving. However, the introduction of roller
milling at the end of the 19th century enabled the pre-
cise separation of the starchy endosperm from
the embryo (germ) and the outer layers (including the
aleurone), which are usually recovered together as the
'bran'. This resulted in the availability of affordable
white bread, previously an expensive luxury, for the
whole population. However, the removal of both the
germ and the aleurone layer means that white flour
has substantially lower contents of iron and zinc than
wholemeal – for example, means of 6.7 mg/kg iron
and 8.4 mg/kg zinc in white flour, compared to
28.2 mg/kg iron and 28.6 mg/kg zinc in the whole-
grain (Tang *et al.* 2008). The contents of iron and zinc
can partially be restored in white flour by enriching it
with wheat germ as in 'patent breads' (Burnett 2005).
In addition, numerous public health campaigns have
been aimed at increasing the consumption of whole-
meal products. Nevertheless, of the total bread-making
flour produced in the UK, <10% is wholemeal
(NABIM 2017).

Roller milling also results in the removal of most of
the anti-nutrient phytic acid, which limits mineral
bioavailability (as discussed below). Phytic acid (inosi-
tol hexakisphosphate) is the storage form of phospho-
rus of plants, which is another important element for
seedling germination and growth.

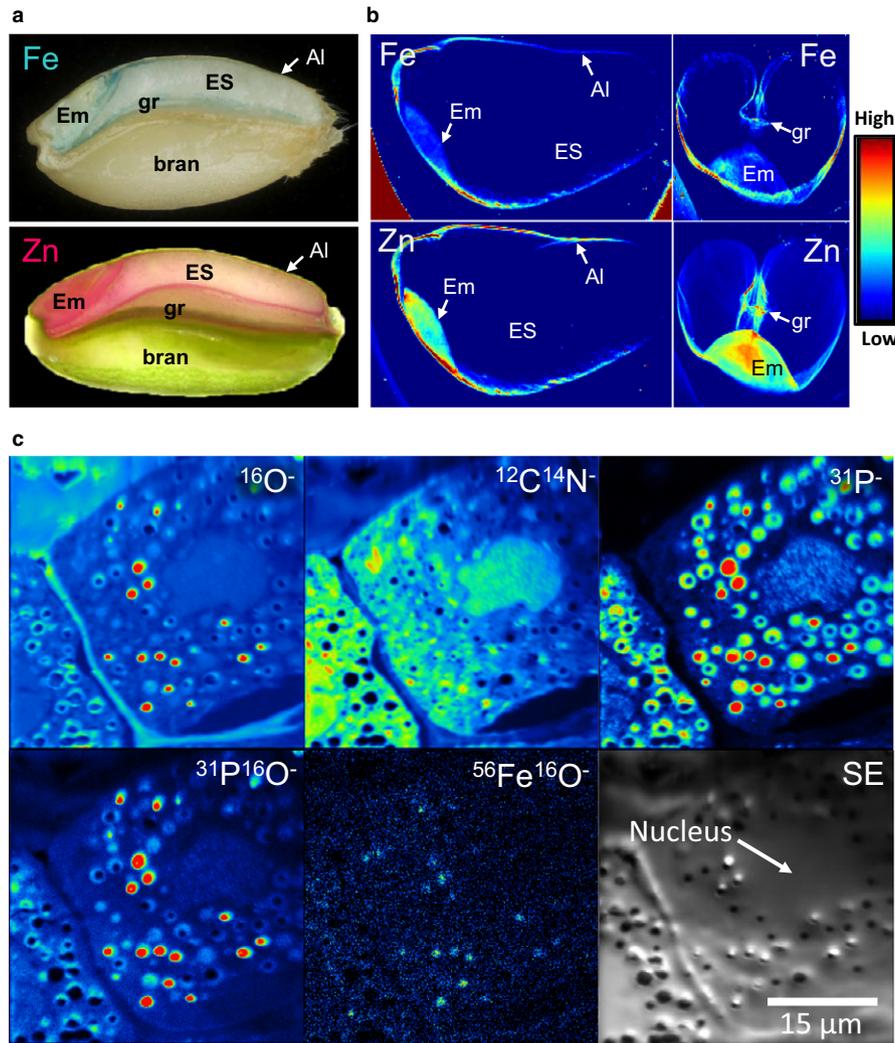


Figure 1 The locations of iron and zinc in wheat grain. (a) Transverse sections through wheat grains, showing the position of the embryo (Em), starchy endosperm (ES), aleurone (Al), groove (gr) and bran and the locations of iron (light blue from staining with Prussian blue in upper image) and zinc (red from staining with dithizone in lower image). (b) Heat map representation of the distribution of iron (Fe) and zinc (Zn) in longitudinal and transverse sections of wheat grain, revealed by X-ray fluorescence. Taken from Neal *et al.* (2013) with permission. Labelling as in Panel (a). (c) NanoSIMS images of an aleurone cell of an immature wheat grain showing localisation of $^{56}\text{Fe}^{16}\text{O}^-$ to the phytin globoids (as indicated by the $^{31}\text{P}^{16}\text{O}^-$ image). White indicates high signal intensity. Taken from Moore *et al.* (2012) with permission.

Naturally occurring iron and zinc compounds and their bioavailability

Iron and zinc are essential cofactors in a wide range of metabolic enzymes. Thus, these elements occur in protein-bound forms in plants and animals. Iron can bind directly to the protein, or as an iron–sulphur cofactor or as haem (Balk & Schaedler 2014). The bioavailability of haem iron is high because it is very stable and is thought to be taken up by a specific transporter in the smaller intestine (Knutson 2017). However, plants contain very little haem iron (<0.1% w/w; Espinas *et al.* 2012) in contrast to animal tissues

(notably meat and liver). Zinc binds to proteins directly, for example, in zinc-finger structures.

Iron and zinc are transported in plants as soluble forms chelated by small organic molecules and reach the developing grain through specialised vascular tissues (xylem and phloem), which extend along the groove of the grain (Fig. 1a). The organic acids citrate and malate facilitate the transport of iron in the xylem while nicotianamine facilitates iron and zinc transport in the phloem and intracellularly (Connorton *et al.* 2017a). Iron–nicotianamine complexes are present in extracts of white wheat flour (Eagling *et al.* 2014a) and nicotianamine enhances the bioavailability of iron

and zinc as demonstrated in mouse studies (Lee *et al.* 2009) and in Caco-2 cells (Eagling *et al.* 2014b).

Plants have the capability to store iron either inside a shell formed by the protein ferritin or in ~~small vesicles~~ ³ vesicles (Connorton *et al.* 2017a). Wheat grains are low in ferritin, but high-resolution imaging techniques such as Nano-SIMS show that iron is localised in small intracellular bodies (Fig. 1c). These bodies also contain phosphate in the form of phytic acid and are called phytin globoids. Phytic acid has a cyclic structure with six phosphate groups, each of which can bind a metal ion such as iron and zinc (Maga 1982). Neal *et al.* (2013) used extended X-ray fluorescence fine structure spectroscopy (EXAFS) to show the presence of iron and zinc complexes in wheat aleurone cells. A further application of X-ray fluorescence, X-ray absorption near-edge structure (XANES) imaging, gives information on the atomic ligands of the metals, confirming that most of the iron is bound to phytic acid in the aleurone (De Brier *et al.* 2016). This colocalisation poses a challenge for human nutrition because mineral–phytate complexes tend to be insoluble with low bioavailability in humans.

Agronomic and selective breeding strategies to increase bioavailable forms of iron and zinc

In addition to improvements in yield, disease resistance and processing quality, there has been growing research interest over the past 20 years in improving the health benefits of cereal crops, including increasing their mineral and vitamin contents (Vasconcelos *et al.* 2017), an approach known as biofortification. However, this has focused on increasing the mineral content in the wholegrain, rather than on the starchy endosperm tissue (which may limit the bioavailability).

There are two main biofortification approaches: agronomy and genetics (including conventional breeding and genetic modification; GM). Using agronomic methods, the zinc content of grain can be increased by simply fertilising the plants with zinc salts; for example, foliar application of ZnSO₄ increased total grain zinc by about 60% (Zhang *et al.* 2012). However, such agronomic practices are less effective for iron, except if combined with increased nitrogen fertilisation (Aciksoz *et al.* 2011) which may not be economically or environmentally acceptable. Conventional breeding has been used by workers at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, India) to develop varieties of sorghum and

pearl millet with increased contents of iron and zinc, and at the International Maize and Wheat Improvement Center (CIMMYT, Mexico) to increase the zinc content of wheat grain (Velu *et al.* 2018).

The zinc biofortified lines from CIMMYT are currently being grown in Pakistan and India and have 20–40% higher zinc concentration and at least comparable grain yield to the best local cultivars (Velu *et al.* 2018). Furthermore, human intervention trials to determine the bioavailability of the zinc in the biofortified lines are currently being carried out in Pakistan (Lowe *et al.* 2018). However, despite a number of research programmes globally, including at CIMMYT, no high iron wheat lines have yet been developed by conventional breeding.

Transgenic strategies to increase bioavailable forms of iron and zinc

A step-change in our ability to biofortify crops has come from a much better understanding of how plants take up and distribute micronutrients, mainly through the identification of genes for mineral transport and the biosynthesis of organic metal chelators. This knowledge has been exploited in modern biotechnology approaches, demonstrating that it is possible to increase iron and zinc levels, not only in the wholegrain but also specifically in the starchy endosperm. In fact, this shows that there is no biological reason that iron and zinc cannot be concentrated in the starchy endosperm and hence white flour. ⁴

The proof-of-concept of transgenic ~~approach~~ approach was initially demonstrated in rice. Increased expression of *NAS3*, one of three genes encoding nicotianamine synthase (NAS), led to a 2.2-fold increase in the concentration of zinc and a 2.9-fold increase in the concentration of iron in the grain (Lee *et al.* 2009). Furthermore, feeding anaemic mice this enriched rice resulted in greater increases in haemoglobin and haematocrit (the volume of red blood cells in blood) compared to when conventional rice was fed. This high bioavailability results from the fact that the starchy endosperm cells do not store phytate. The initial transgenic work on NAS in rice led to similar studies in other cereals including wheat (*e.g.* Masuda *et al.* 2009; Zheng *et al.* 2010; Johnson *et al.* 2011; Singh *et al.* 2017). The advantage of increasing nicotianamine levels is that it leads to increases in both iron and zinc because it serves as a chelator for both metals in their ionic forms.

By contrast, redirecting minerals into the starchy endosperm cells by overexpressing metal transporter

genes leads to increases in single minerals, due to the high specificity of metal transporters, unless several genes are overexpressed together. For example, expression of the barley Metal Tolerance Protein 1 (*HvMTP1*), under the control of a starchy endosperm-specific promoter, significantly increased the zinc content in the endosperm of barley grains (Menguier *et al.* 2018), while expression of a wheat Vacuolar Iron Transporter (*TaVIT2*) using a similar promoter more than doubled the iron content of the white flour fraction (Connorton *et al.* 2017b). This is illustrated in Fig. 2, which compares the contents of iron, zinc and phosphorus in bran and flour fractions of a *TaVIT2* transgenic line with control wheat grain milled on a laboratory roller mill. It is notable that the increased content of iron in the white flour (break and reduction) fractions in the transgenic line is not accompanied by an increase in phosphorus, showing that iron can accumulate in the endosperm without being associated with phytic acid.

More recently, Yu *et al.* (2018) have shown that preventing iron storage in the vacuoles, while at the same time overexpressing the iron storage protein ferritin specifically in the starchy endosperm cells, greatly increased iron in polished rice.

Hence, it is now accepted that transgenesis can be used to increase the contents of bioavailable iron and zinc in the starchy endosperm of cereals (white flour of wheat and polished white rice) by several-fold, by redirecting mineral transport and/or providing a sink to sequester the iron.

Post-harvest improvements of iron and zinc bioavailability

It is probable that modern breeding approaches discussed above will be combined with novel processing approaches to increase mineral content availability in wheat-based foods, ranging from wholemeal to white flour products. The two most promising of these are

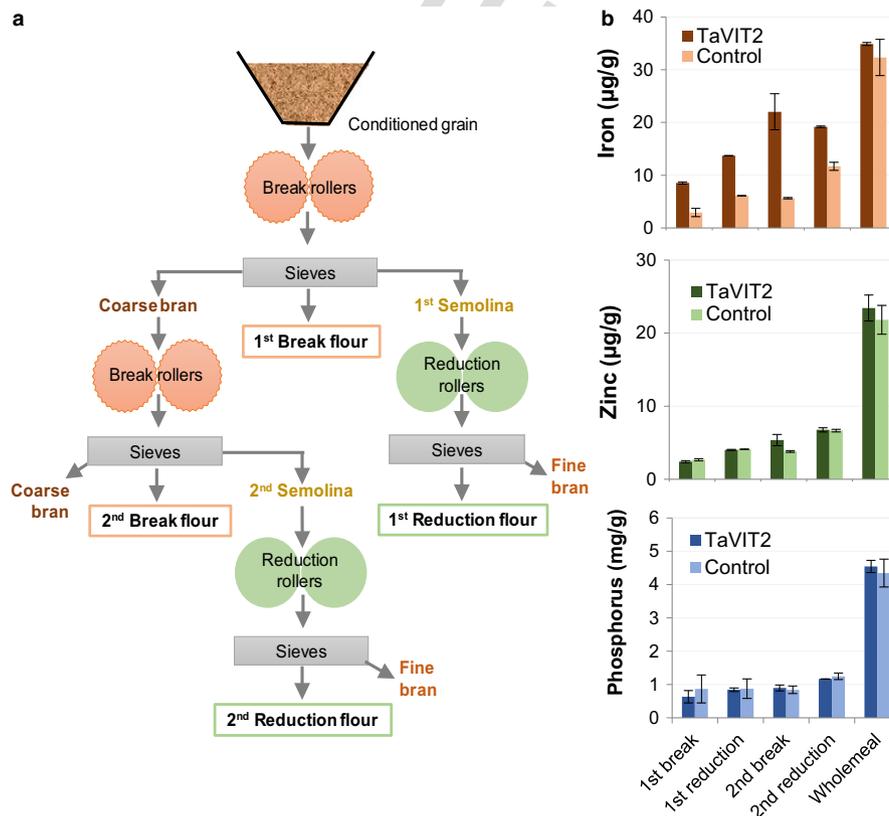


Figure 2 The contents of iron, zinc and phosphorus in white flour and wholemeal fractions from a transgenic wheat line expressing a vacuolar iron transporter (*TaVIT2*) and control wheat grain. (a) Milling scheme used to prepare white flour fractions (breaks 1 and 2, reductions 1 and 2) from the transgenic line *TaVIT2* and control wheat grain using a Chopin CD1 laboratory mill. This scheme is a simplified version of the industrial roller milling process, with the first break and reduction flours being purer than the second break and reduction flours. (b) The contents of iron, zinc and phosphorus in white flour fractions (prepared using the scheme in part a) and wholemeal flour. Mineral contents were determined by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) analysis. Bars indicate mean \pm standard error of the mean (SEM) of two technical replicates.

mechanical treatments and fermentation. Mechanical treatments, particularly micro-grinding, have been discussed in a recent article in this journal (Aslam *et al.* 2018). Plant cell walls are resistant to digestion in the gastro-intestinal tract and therefore mineral bioaccessibility from wheat aleurone cells may be limited (Latunde-Dada *et al.* 2014; Edwards *et al.* 2015).

5 Enzymatic treatment and micro-milling techniques designed to disrupt the aleurone cell walls enhance the release of iron from wheat flour during *in vitro* digestion and increase iron uptake by intestinal epithelial cells (Latunde-Dada *et al.* 2014). This suggests that disruption of the aleurone cell walls may be an effective approach to increase iron bioavailability from wheat products.

Many microorganisms secrete phytase enzymes, which can release minerals from phytate complexes, particularly microorganisms present in sourdough systems (Katina *et al.* 2015; Rodriguez-Ramiro *et al.* 2017). Hence sourdough wholegrain products may have increased mineral bioavailability. However, whereas this approach may increase mineral bioavailability in foods made from wholegrain and high extraction flours, it is not relevant to white flour products which are dominant in most countries.

Future perspectives

Although transgenic strategies provide exciting opportunities to make dramatic increases in the contents and bioavailabilities of minerals in white flour products, it must be borne in mind that transgenic crops have limited acceptability by consumers and regulatory bodies, particularly in the European Union but also in many less developed countries. Furthermore, the recent (July 2018) ruling from the European Court of Justice that gene-edited crops, which do not contain foreign DNA, should be considered genetically modified organisms (GMOs) (<http://curia.europa.eu/juris/celex.jsf?celex=62016CJ0528&lang1=en&type=TEXT&ancre>) suggests that restrictions on growth and marketing are unlikely to be relaxed in the near future.

Although conventional mutagenesis remains outside GM legislation, most mutations result in loss, or reduced, gene expression and the application of mutagenesis to biofortification is therefore a challenge. Nevertheless, this is likely to be the most promising route for ~~deploying~~ biofortified wheat in the future. Elucidation of the pathways and mechanisms of iron and zinc transport and deposition in the developing grain should identify genes encoding key transporters, or other factors, that can be downregulated or

switched off, to redirect minerals into the starchy endosperm. This will be facilitated by the availability of comprehensive libraries of wheat mutants (Krasileva *et al.* 2017). Combined with conventional biofortification and innovative processing this should provide increased mineral bioavailability in a range of wheat products, from white flour to wholemeal.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

References

- Aciksoz SB, Yaziki A, Ozturk L *et al.* (2011) Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilisers. *Plant and Soil* **349**: 215–25.
- Aslam MF, Ellis PR, Berry SE *et al.* (2018) Enhancing mineral bioavailability from cereals: current strategies and future perspectives. *Nutrition Bulletin* **43**: 184–8.
- Balk J & Schaedler TA (2014) Iron cofactor assembly in plants. *Annual Review of Plant Biology* **65**: 125–53.
- Bastow EL, Garcia de la Torre VS, Maclean AE *et al.* (2018) Vacuolar iron stores gated by NRAMP3 and NRAMP4 are the primary source of iron in germinating seeds. *Plant Physiology* **177**: 1267–76.
- Bates B, Lennox A, Prentice A *et al.* (eds) (2014) *National Diet and Nutrition Survey: Results from Years 1–4 (combined) of the Rolling Programme (2008/2009 – 2011/2012)*. Public Health England: London, UK.
- Brown KH, Hambidge KM, Ranum P *et al.* (2010) Zinc fortification of cereal flours: current recommendations and research needs. *Food and Nutrition Bulletin* **31**(1 Suppl): S62–74.
- Burnett J (2005) Brown is best. *History Today* **55**: 52–4.
- Connorton JM, Balk J & Rodriguez-Celma J (2017a) Iron transport in plants – a brief overview. *Metallomics* **9**: 813–23.
- Connorton JM, Jones ER, Rodriguez-Ramiro I *et al.* (2017b) Wheat vacuolar iron transporter TaVIT2 transports Fe and Mn and is effective for biofortification. *Plant Physiology* **174**: 2434–44.

- De Brier N, Gomand SV, Donner E *et al.* (2016) Element distribution and iron speciation in mature wheat grains (*Triticum aestivum* L.) using synchrotron X-ray fluorescence near-edge structure (XANES) imaging. *Plant Cell and Environment* **39**: 1835–47.
- Eagling T, Neal AL, McGrath SP *et al.* (2014a) Distribution and speciation of iron and zinc in grain of two wheat genotypes. *Journal of Agricultural and Food Chemistry* **62**: 708–16.
- Eagling T, Wawer AA, Shewry PR *et al.* (2014b) Iron bioavailability in two commercial cultivars of wheat: comparison between whole-grain and white flour and the effects of nicotianamine and 2'-deoxymugineic acid on iron uptake into Caco-2 cells. *Journal of Agricultural and Food Chemistry* **62**: 10320–5.
- Edwards CH, Grundy MM, Grassby T *et al.* (2015) 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**: 791–800.
- Espinás NA, Kobayashi K, Takahashi S *et al.* (2012) Evaluation of unbound free heme in plant cells by differential acetone extraction. *Plant and Cell Physiology* **53**: 1344–54.
- Heath AL & Fairweather-Tait SJ (2002) Clinical implications of changes in the modern diet: iron intake, absorption and status. *Best Practice in Research in Clinical Haematology* **15**: 225–41.
- Johnson AAT, Kyriacou B, Callahan DL *et al.* (2011) Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS ONE* **6**: e24476.
- Katina K, Arendt E, Liukkonen K-H *et al.* (2015) Potential of sourdough for healthier cereal products. *Trends in Food Science and Technology* **16**: 104–12.
- Knutson MD (2017) Iron transport proteins: gateways of cellular and systemic iron homeostasis. *Journal of Biological Chemistry* **292**: 12735–43.
- Krasileva V, Vasquez-Gross HA, Howell T *et al.* (2017) Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences of the United States of America* **114**: E913–21.
- Latunde-Dada GO, Li X, Parodi A *et al.* (2014) Micromilling enhances iron bioaccessibility from wholegrain wheat. *Journal of Agricultural and Food Chemistry* **62**: 11222–7.
- Lee S, Jeon US, Kim Y-K *et al.* (2009) Iron fortification of rice seeds through activation of the *nicotianamine synthase* gene. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 22014–9.
- Lowe NM, Khan MJ, Broadley MR *et al.* (2018) Examining the effectiveness of consuming flour made from agronomically biofortified wheat (Zincol-2016/NR-421) for improving Zn status in women in a low-resource setting in Pakistan: study protocol for a randomised, double-blind, controlled cross-over trial (BiZiFED). *British Medical Journal Open* **8**: e021364.
- Maga JA (1982) Phytate: its occurrence, food interactions, nutritional significance and methods of analysis. *Journal of Agricultural and Food Chemistry* **30**: 1–9.
- Masuda H, Usuda K, Kobayashi T *et al.* (2009) Overexpression of the barley nicotianamine synthase gene *HvNAS11* increased iron and zinc concentrations in rice grains. *Rice* **2**: 155–66.
- Menguer PK, Vincent T, Miller AJ *et al.* (2018) Improving zinc accumulation in cereal endosperm using HvMTP1, a transition metal transporter. *Plant Biotechnology Journal* **16**: 63–71.
- Moore KL, Zhao F-J, Gritsch C *et al.* (2012) Localisation of iron in wheat grain using high resolution secondary ion mass spectrometry. *Journal of Cereal Science* **55**: 183–7.
- NABIM (2017) *UK Flour Milling Industry 2017. The National Association of British and Irish Millers*: ????.
- Neal AL, Geraki K, Borg S *et al.* (2013) Iron and zinc complexation in wild-type and ferritin-expressing wheat grain: implications for mineral transport into developing grain. *Journal of Bioinorganic Chemistry* **18**: 557–70.
- Ritchie H, Roser M (2018) *Diet compositions*. Available at: <https://ourworldindata.org/diet-compositions> (accessed 1 November 2018).
- Rodriguez-Ramiro I, Brearley CA, Bruggaber SFA *et al.* (2017) Assessment of iron bioavailability from different bread making processes using an *in vitro* intestinal cell model. *Food Chemistry* **228**: 91–8.
- Singh SP, Keller B, Gruissem W *et al.* (2017) Rice *NICOTIANAMINE SYNTHASE 2* expression improves dietary iron and zinc levels in wheat. *Theoretical and Applied Genetics* **130**: 283–92.
- Tang J, Zou C, He Z *et al.* (2008) Mineral element distributions in milling fractions of Chinese wheats. *Journal of Cereal Science* **48**: 821–8.
- Vasconcelos MW, Gruissem W & Bhullar NK (2017) Iron biofortification in the 21st century: setting realistic targets, overcoming obstacles, and new strategies for healthy nutrition. *Current Opinion in Biotechnology* **44**: 8–15.
- Velu G, Singh R, Balasubramanian A *et al.* (2018) Reaching out to farmers with high zinc wheat varieties through public-private partnerships – an experience from Eastern-Gangetic plains of India. *Advances in Food Technology and Nutritional Sciences* **1**: 73–5.
- WHO (2013) *World Health Report: Research for Universal Health Coverage*. World Health Organization: Geneva, Switzerland.
- WHO (2015) *The Global Prevalence of Anaemia in 2011*. World Health Organization: Geneva, Switzerland.
- Young TE & Gallie DR (2000) Programmed cell death during endosperm development. *Plant Molecular Biology* **44**: 283–301.
- Yu T-Y, Gruissem W & Bhullar NK (2018) Targeting intracellular transport combined with efficient uptake and storage significantly increases grain iron and zinc levels in rice. *Plant Biotechnology Journal* ????: ???–???. <https://doi.org/10.1111/ppi.12943>.
- Zhang Y-Q, Sun Y-X, Ye Y-L *et al.* (2012) Zinc biofortification of wheat through fertilizer applications in different locations of China. *Field Crops Research* **125**: 1–7.
- Zheng L, Cheng Z, Ai C *et al.* (2010) Nicotianamine, a novel enhancer of rice iron bioavailability to humans. *PLoS ONE* **5**: e10190.