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Nutrient stability and bioavailability of biofortified crops along the value chain

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Biofortification Workshop
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Outline

- The what, why and how of biofortification
- Typically biofortified nutrients and crops
- Factors influencing nutrient stability
- Determinants of nutrient bioavailability

What is biofortification?

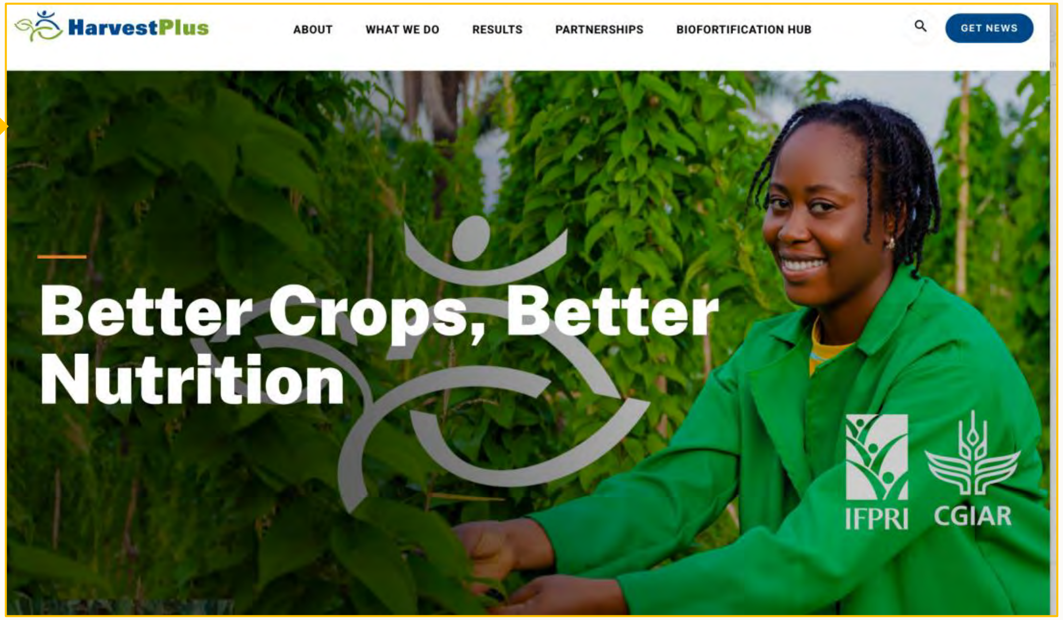
- Originally defined as the enrichment of **staple crops** with **essential micronutrients** to **combat hidden hunger**

- Biofortification has evolved into a broader strategy to enhance **nutritional quality and health potential** of **crops, feeds and food ingredients** to improve diets

How are foods biofortified?

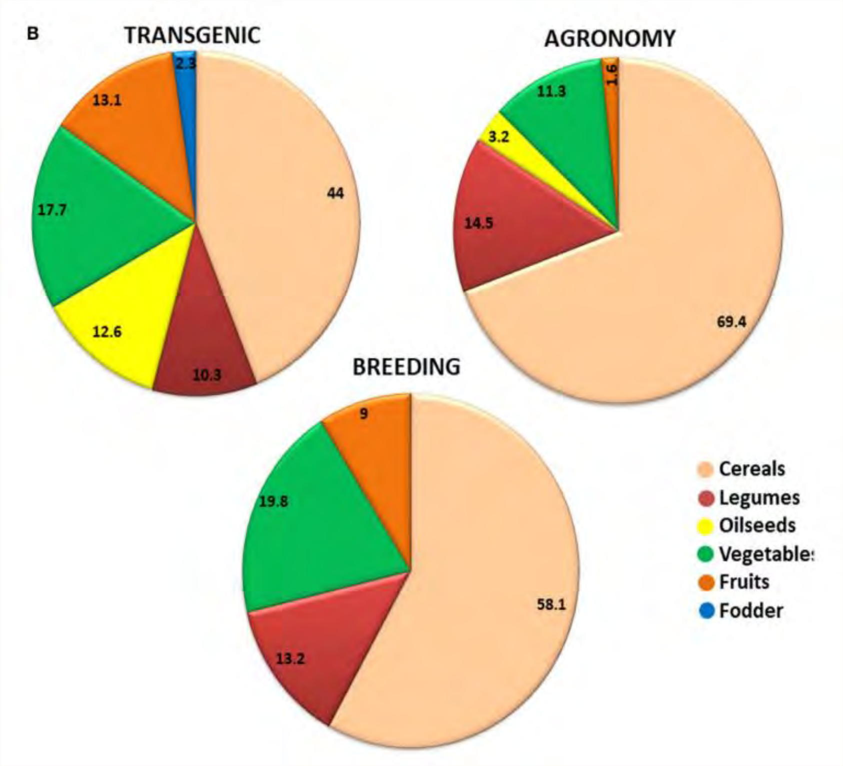
Biofortification

- Conventional breeding
- Agronomic biofortification
- Modern biotechnology



Typical biofortified nutrients and crops

- Vitamin A (T, B)
- Folic acid (T)
- Multivitamins (T)
- Iron, zinc (T, B)
- Iodine (T, A)
- Selenium (T, A)
- Calcium (T, B, A)
- Phytase activity/phytate (T, B)
- Essential amino acids (lysine, methionine, tryptophan) (T, B)
- Polyphenols/antioxidants (T, B)
- Unsaturated fatty acids (T)
- Starch (T)



Transgenic (T), Breeding (B), Agronomic (A)

Other food ingredients being biofortified: Alternative protein sources (e.g., fungal mycelium, microalgae, edible insects, …)

Garg et al. 2018. Frontiers in Nutr

Why consider nutrient stability and bioavailability

- Biofortification occurs at the beginning of the value chain
- Foods undergo multiple processes along the value chain
- Biofortification targets must account for nutrient transformations along the entire value chain, not just levels at harvest

Why consider nutrient stability and bioavailability

Biofortified crop → **Post-harvest handling** → **Food processing** → **Retail** → **End-user**

Biofortified crop
Nutrient content
& localisation

Post-harvest handling
drying, sorting,
milling, storage

Food processing
heat, mechanical
processes,
formulation

Retail
packaging,
transport,
storage,
distribution

End-user
home storage
washing,
peeling,
cooking,

Nutrient stability/retention

What determines nutrient stability/retention

- Nutrient stability/retention depends on **chemical nature** of the nutrient and **localisation** within the food matrix

- Minerals are chemically stable so are mainly lost in processes with mass transfer

- Vitamins and other nutrients are chemically labile and susceptible to heat, oxygen, light, pH

Minerals (e.g., Iron, zinc, calcium…)

- Minerals are chemically stable, not destroyed by heat, light, pH

- Do not degrade during storage

- Losses occur mainly due to removal or leaching

  - Milling, decortication: biggest losses (polishing rice reduces iron by ~50% and Zn by 20-40%), **extraction rate** is critical
  - Soaking/steeping: diffusion of minerals into water (if discarded)
  - Boiling: loss to cooking water (if discarded)
  - Dry vs. wet processes: dry processes retain more minerals

Vitamins & others

- Vitamins are chemically labile
  - Most losses occur via thermal degradation, oxidation and light exposure
- **Heat**: boiling, frying, roasting, extrusion, drying losses vary by vitamin and matrix
  - Heating proteins in the presence of reducing sugars can lead to losses of lysine (Maillard reaction)
- **Oxygen**: oxidation of vitamins esp. carotenoids during storage
- **Light**: photodegradation of B-vitamins and carotenoids
- **Moisture/humidity**: accelerates enzymatic degradation
- **Matrix disruption**: cutting, mashing, can increase exposure to oxygen

Micronutrient retention of biofortified foods

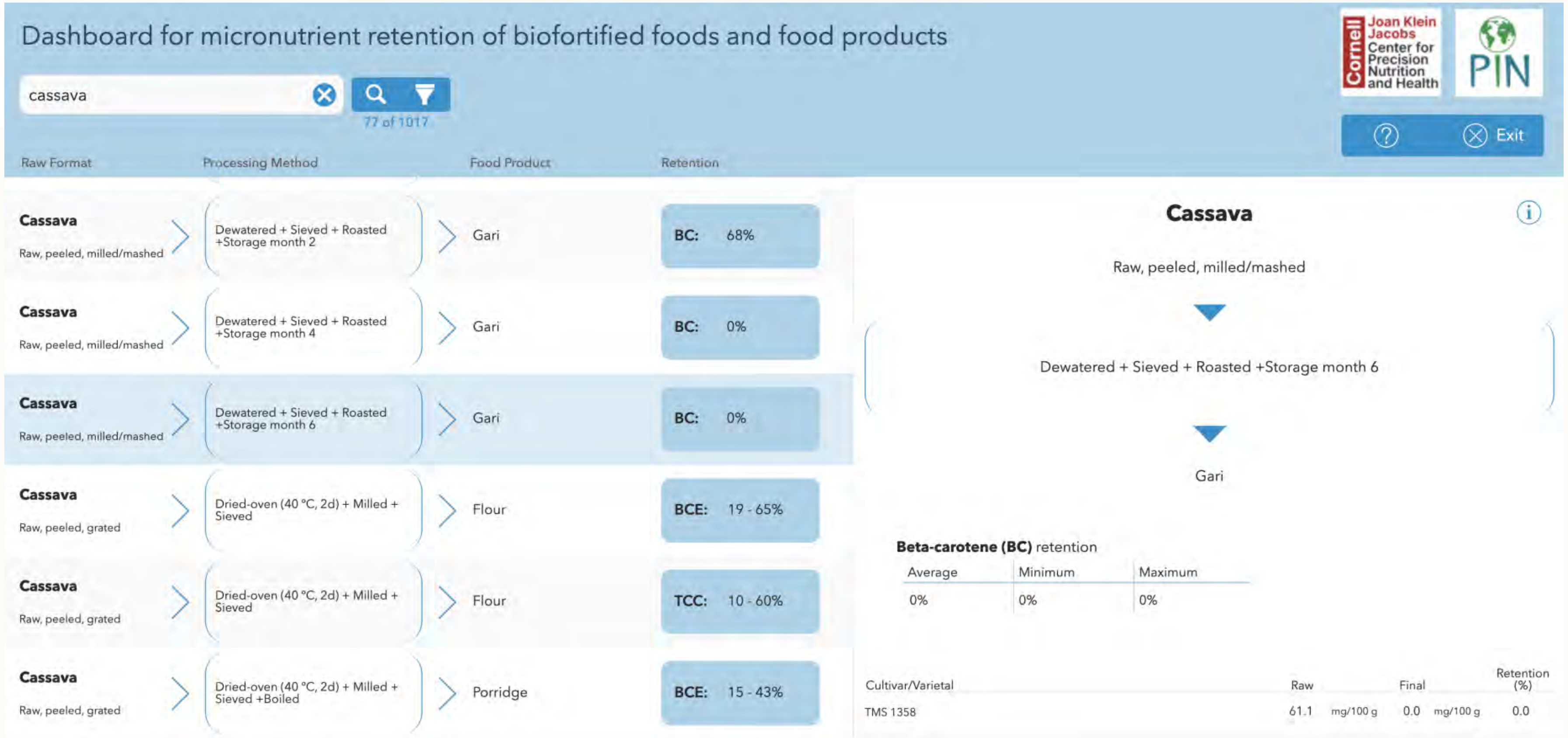
Article | Open access | Published: 09 November 2023

A systematic review of the impacts of post-harvest handling on provitamin A, iron and zinc retention in seven biofortified crops

Samantha L. Huey, Elsa M. Konieczynski, Neel H. Mehta, Jesse T. Krisher, Arini Bhargava, Valerie M. Friesen, Mduduzi N. N. Mbuya, Eva C. Monterrosa, Annette M. Nyangaresi & Saurabh Mehta

Nature Food 4, 978–985 (2023) | Cite this article

https://www.cpnh.cornell.edu/mn-retention-db



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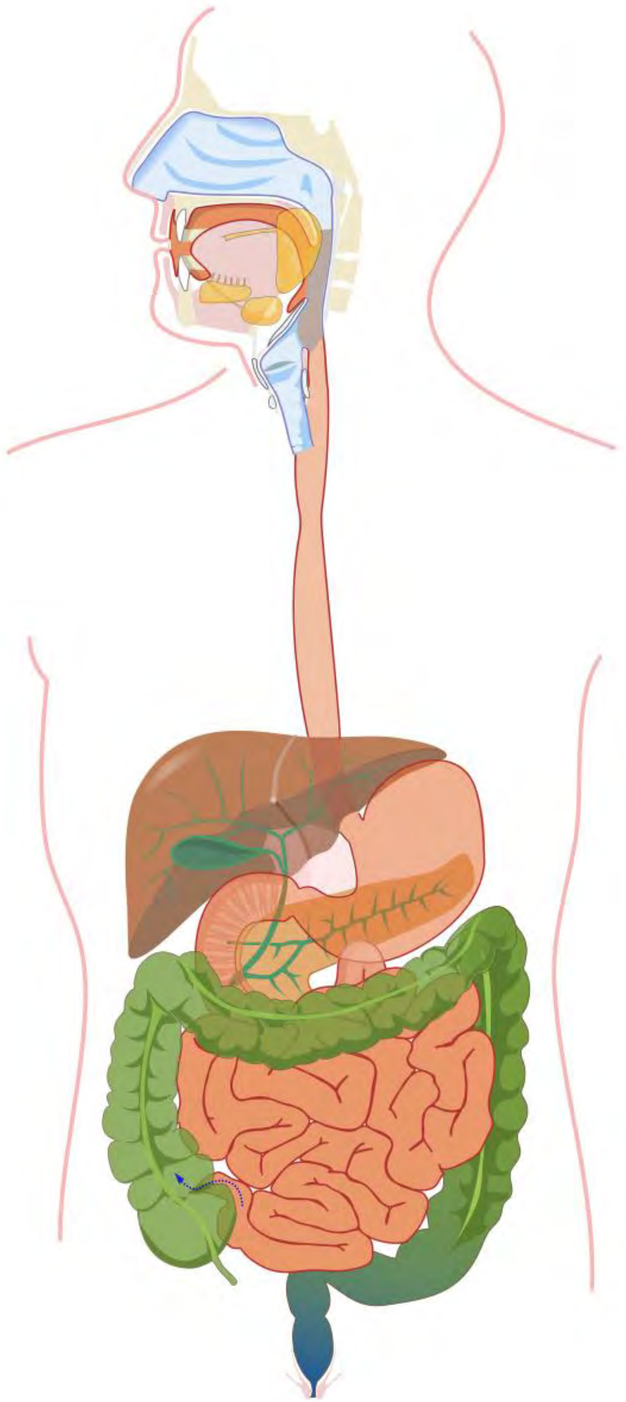
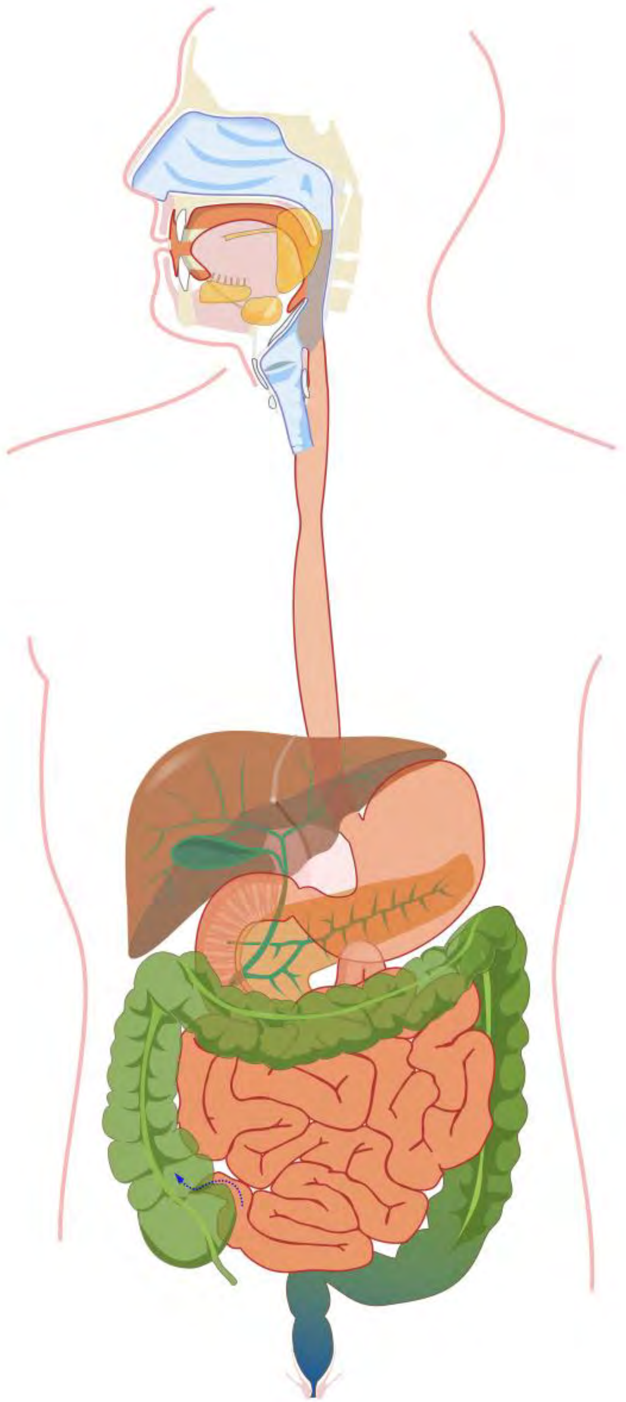
Bioavailability



Bioavailability

- The proportion of ingested nutrient available for physiological processes in the body

- Gross nutrient ingested > bioavailable nutrient

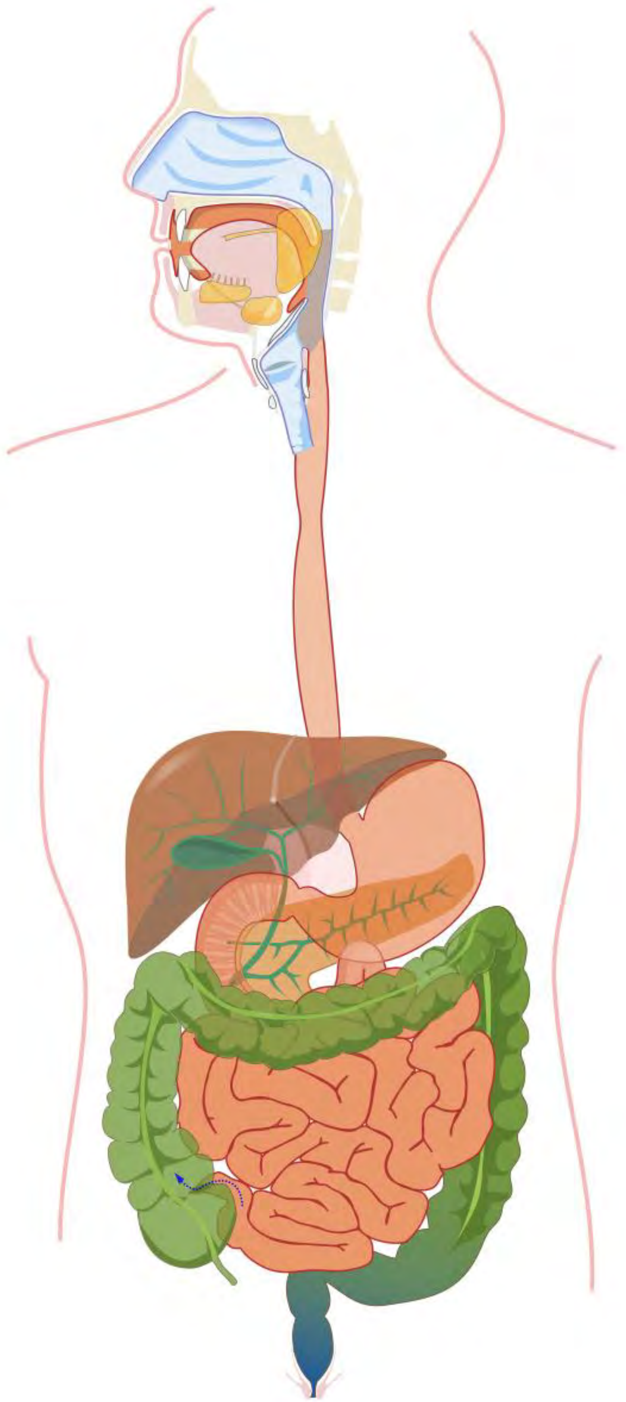


Determinants of bioavailability

- Food structure
  - Cell wall intactness and permeability

- Digestion and absorption inhibitors
  - Phytate, oxalates
  - Tannins (and some types of polyphenols)
  - Protease inhibitors
  - Lectins
  - Goitrogens
  - Fibre

- Nutrient localisation

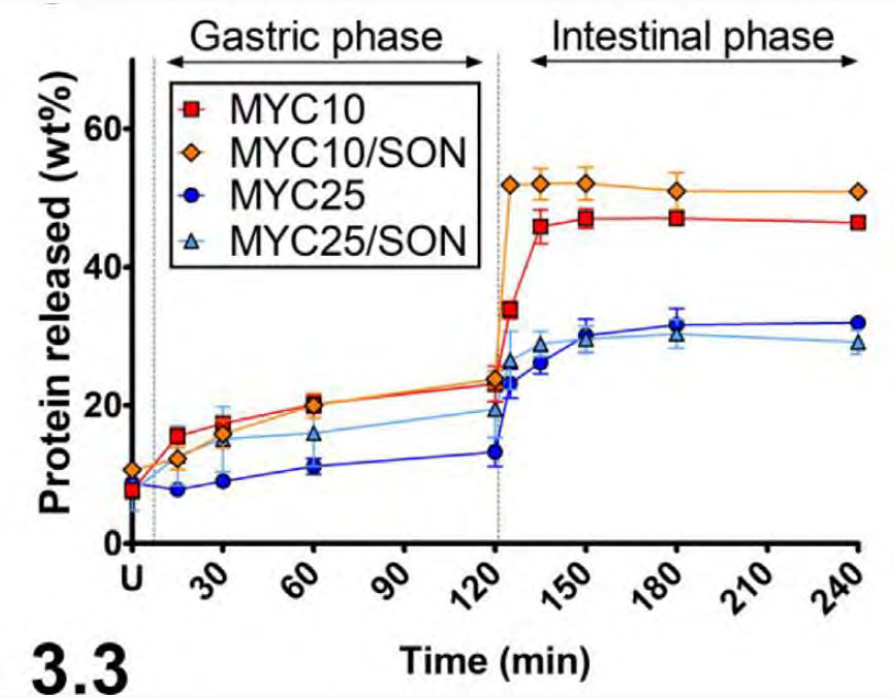


Cell wall intactness and permeability

- Cotyledon cell walls of pulses and beans are resistant to upper gastrointestinal digestion
  - The majority of nutrients in beans are enclosed in cotyledon cells
- Some aleurone cells in wheat can evade gastrointestinal digestion[1]
- Fungal cells remain intact during gastrointestinal digestion
  - Permeability to enzymes responsible for nutrient hydrolysis[2]

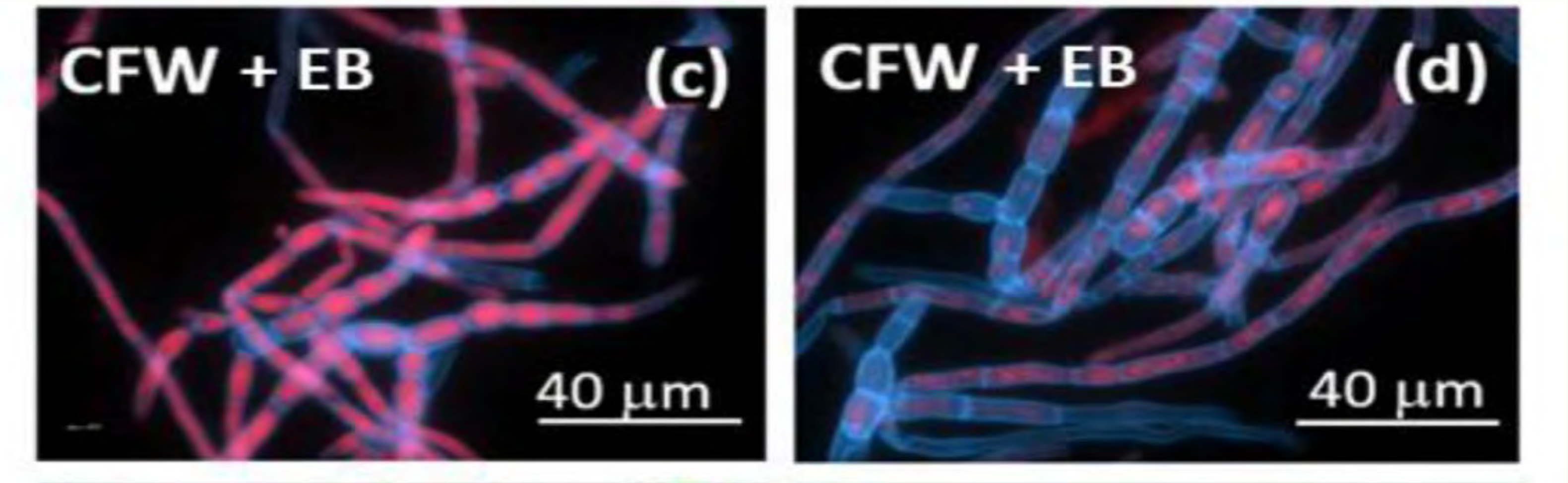
[1]Latunde Dada et al. 2014. J Agric Food Chem; 62:11222−7
[2]Colossimo et al. 2020. Food Chemistry; 330: 127252

Cell wall intactness and permeability



Gastric

Intestinal



Calcofluor white (CFW) and Evans blue (EB) stain used to stain protein red for fluorescence microscopy

- In vitro digestion of mycoprotein fungal mycelium shows intact cells at the end of intestinal digestion
- Porosity/permeability of cells to proteases critical
- Cell walls were resistant to proteases and physical disruption methods (sonication)

Colossimo et al. 2020. Protein bioaccessibility from mycoprotein hyphal structure: In vitro investigation of underlying mechanisms. Food Chemistry

Cell wall intactness

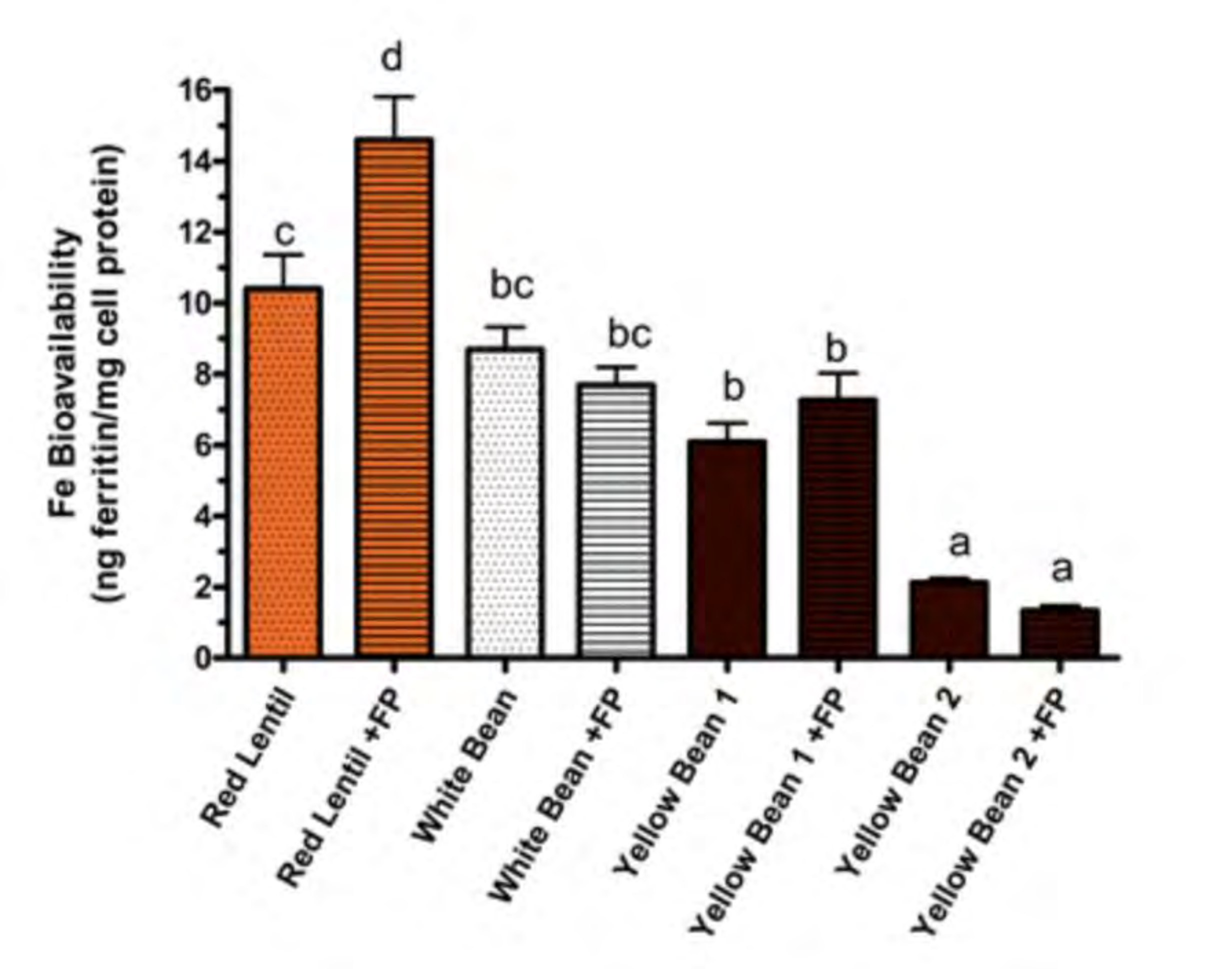


Table 1 Description and source of samples analyzed in the present study

Bean variety	Fe concentration (μg g^{-1})	Bean image	Source (reference where applicable)
White (navy) bean	76		Merlin variety, from Provita, Inc. Grown in 2013 at the Saginaw Valley Research Farm, Richville, MI
Yellow bean 1 (fast cooking, 18.2 min)	67		Dr Karen Cichy, USDA, Michigan State University. From Montcalm location 2015 harvest. AD0P521 Cebo Cela variety
Yellow bean 2 (slow cooking; 35.4 min)	56		Dr Karen Cichy, USDA, Michigan State University. From Montcalm location 2015 harvest. ADP0468, PI527538 variety
Dehulled red lentils	57		Crop Development Center at University of Saskatchewan, Saskatoon, Canada. CDC Robin variety

Iron bioavailability of beans with/without rupture of cotyledons using high pressure French press treatment (FP)

Glahn et al. 2016. The cotyledon cell wall and intracellular matrix are factors that limit iron bioavailability of the common bean. Food Funct.

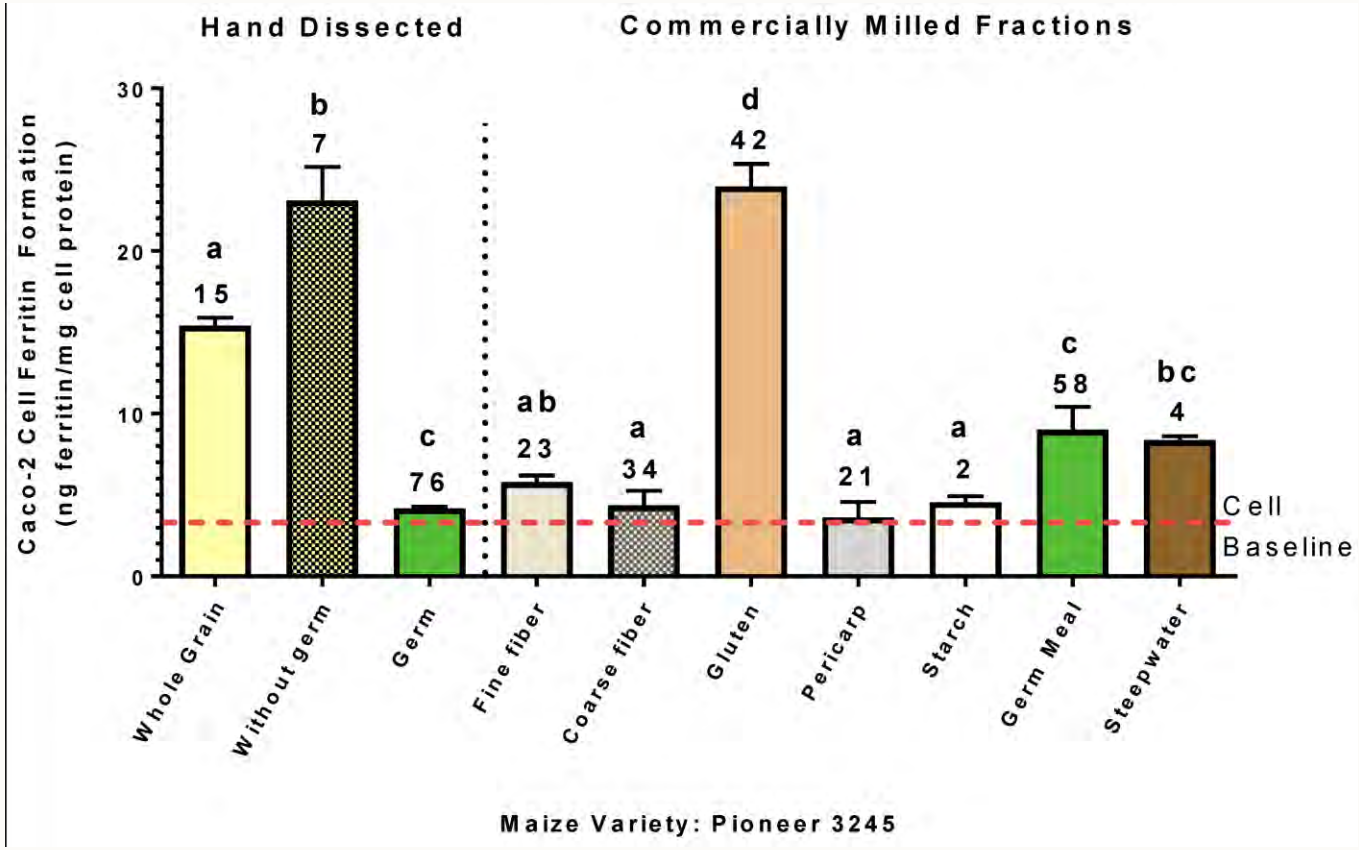
Digestion and absorption inhibitors & their localisation

- Fe & Zn are mostly concentrated in the **aleurone layer** and **embryo** of mature grain
  - Germ fraction of maize contain 27-54% total grain iron
  - Aleurone and scutellum fraction of wheat contain 75-80% of total grain iron

- Present in low levels in the starchy endosperm which is most widely consumed

- Fe & Zn largely present as complexes with **phytic acid**

Digestion and absorption inhibitors & their localisation

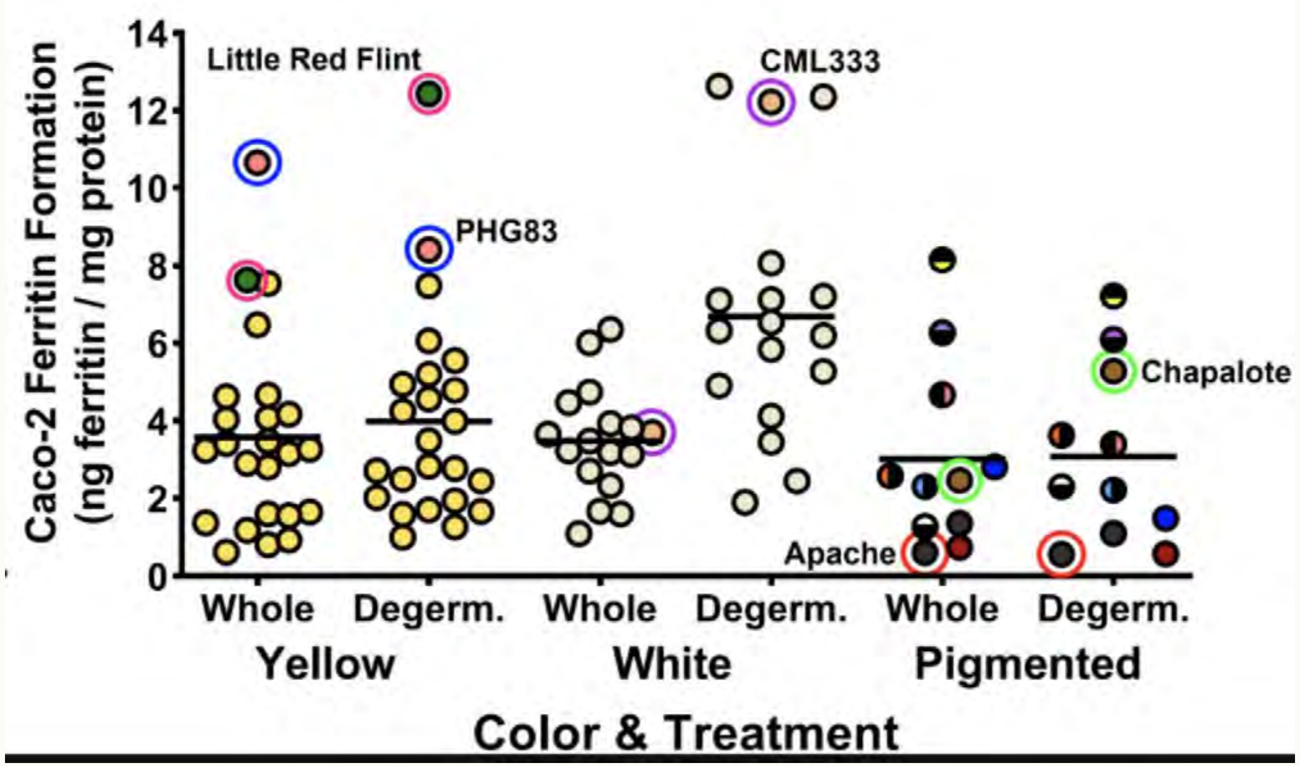


Milling fractions differ widely in iron bioavailability:

- Germ and outer grain layers show low iron uptake, whereas endosperm-rich fractions (e.g., gluten) show the highest
- Co-localisation of iron and phytate in germ/bran strongly limit absorption

Glahn et al. 2019. Germ fraction inhibits iron bioavailability of maize. Nutrients

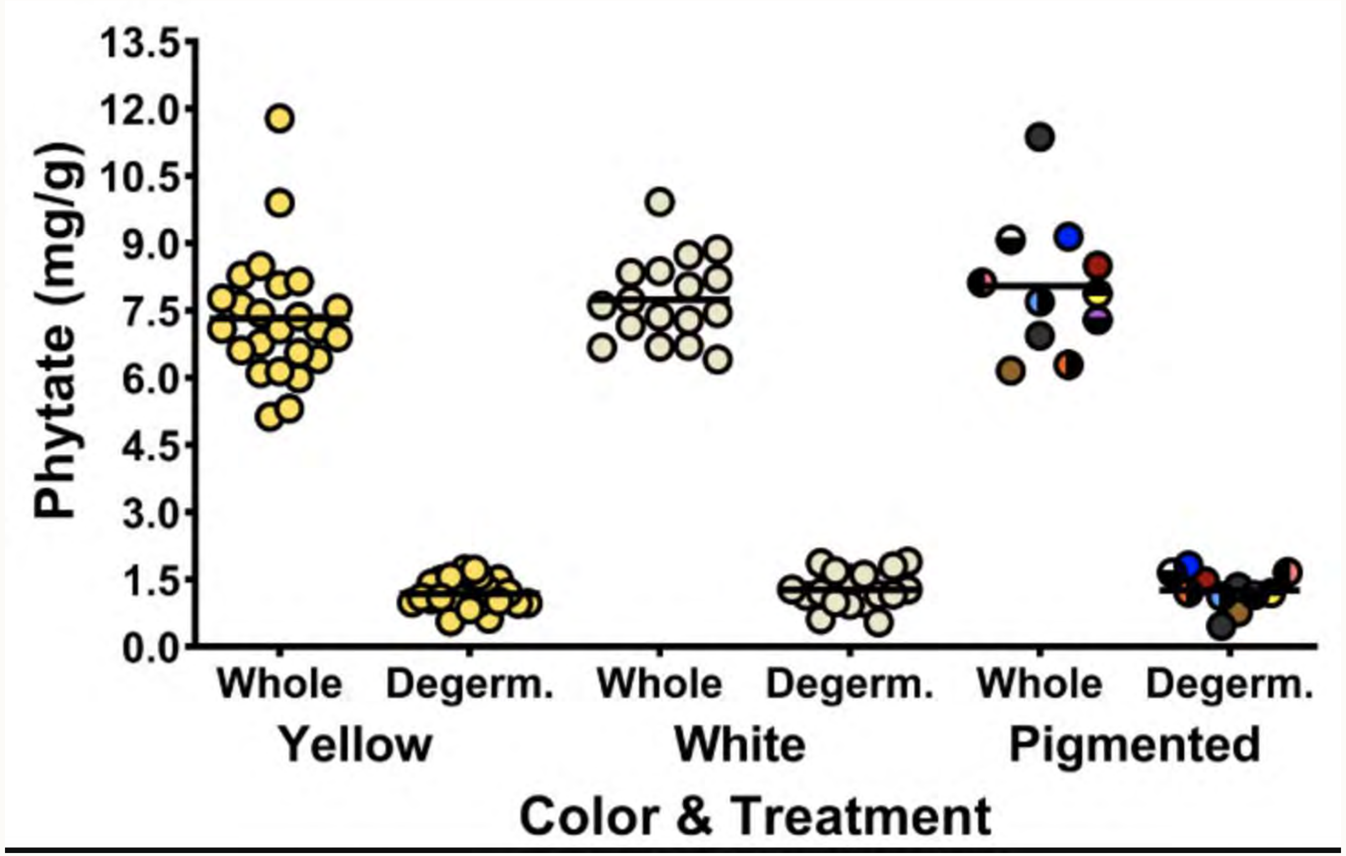
Digestion and absorption inhibitors & their localisation



- Increase in iron uptake after degerming depends on maize variety
- Substantial in white maize varieties
- Not consistent in yellow and pigmented varieties
- Presence of both **phytate** and **polyphenols** in coloured varieties!

Keigler et al. 2023. Iron bioavailability of maize after removing the germ fraction. Front Plant Sci.

Digestion and absorption inhibitors & their localisation

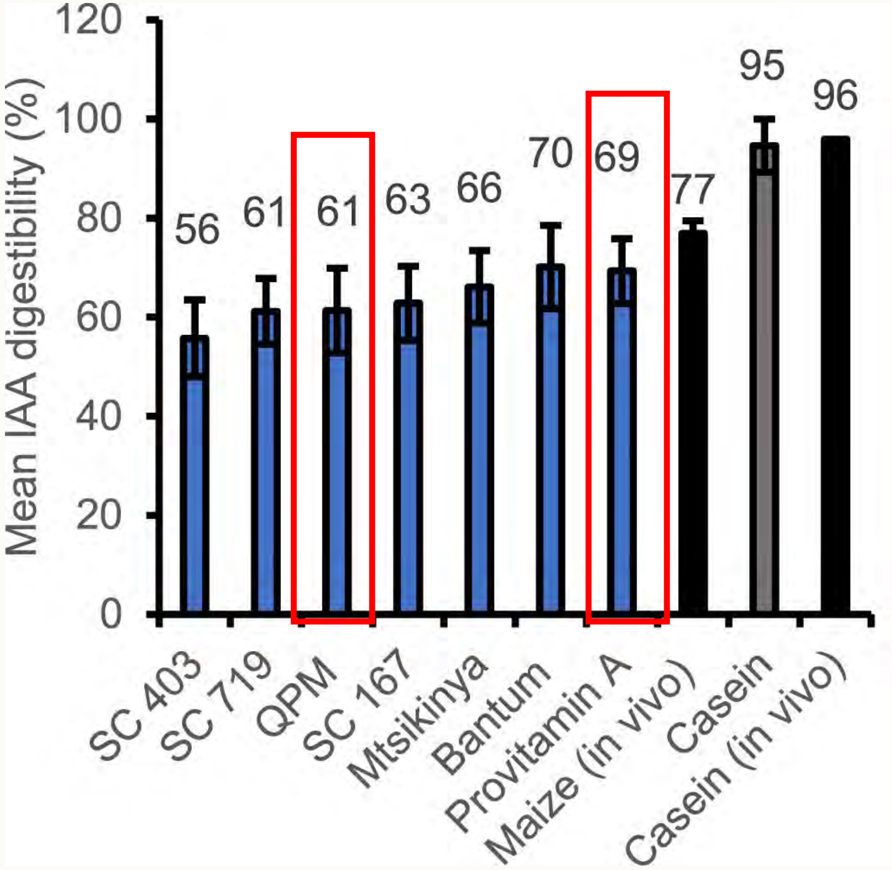


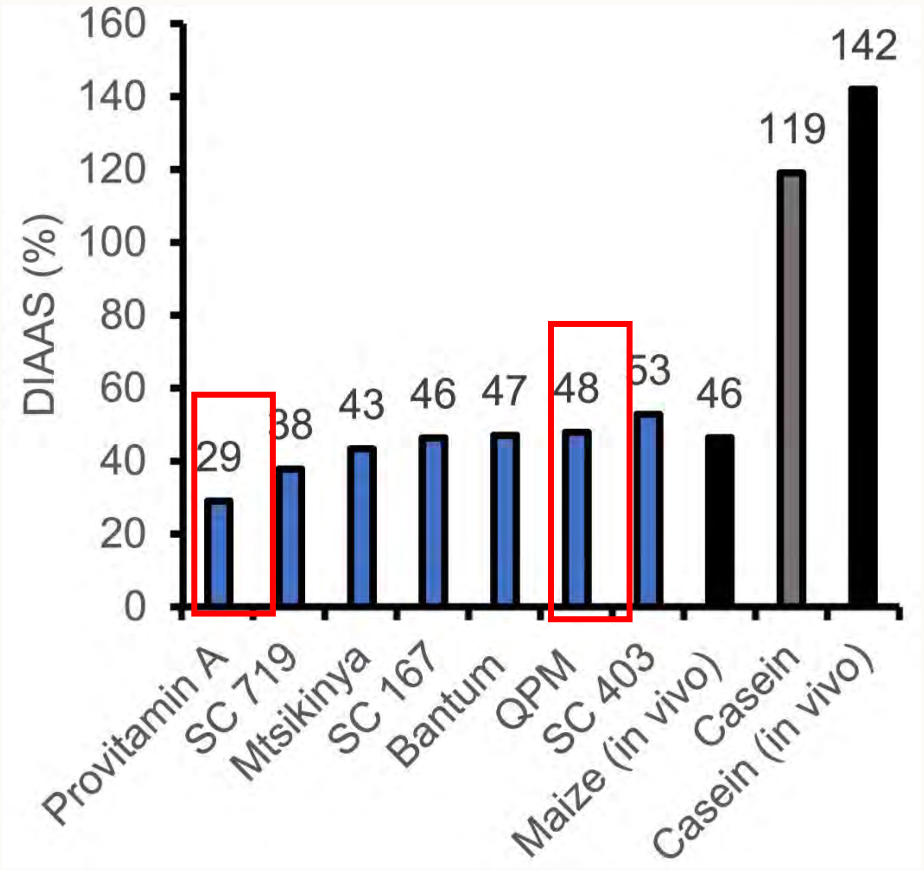
- Co-localisation of iron and phytate in germ/bran strongly limit absorption
- Removal of **phytate alone** in pigmented varieties not sufficient to increase iron uptake

Keigler et al. 2023. Iron bioavailability of maize after removing the germ fraction. Front Plant Sci.

Digestion and absorption inhibitors & their localisation

Comparison of the protein digestibility and quality of biofortified and maize landraces in Malawi





IAA: Indispensable amino acids

DIAAS: digestible indispensable amino acid score

Removal of bran fraction can increase protein digestibility but also remove proteins!

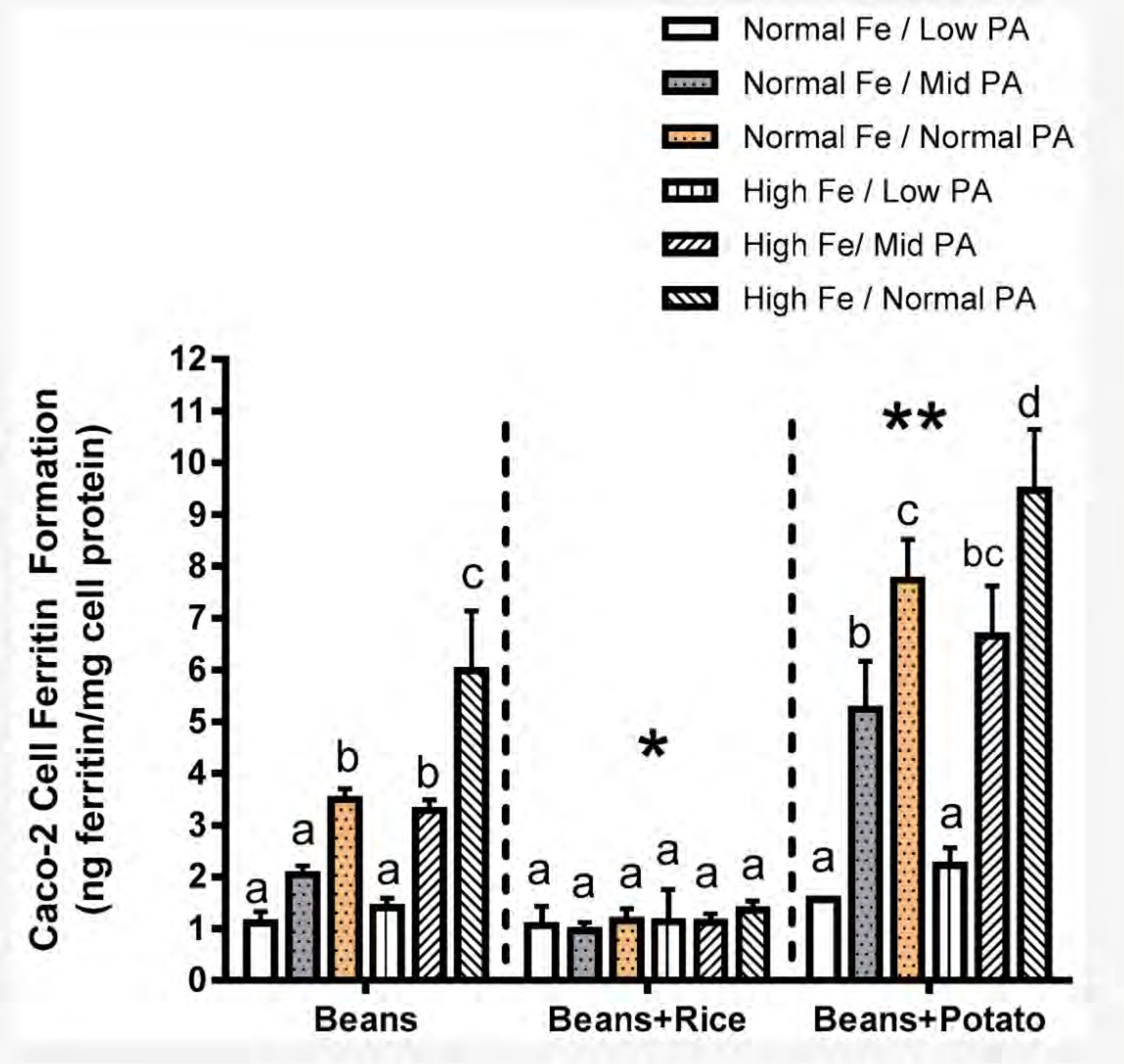
Muleya et al. 2023. In vitro determination of the protein quality of maize varieties cultivated in Malawi using the INFOGEST method. Heliyon.

Bioavailability of meal/diet should be ultimately considered

- Bioavailability is important to consider at ingredient level
- But ultimately, the diet/meal influences overall bioavailability especially for minerals such as Fe, Zn, Ca and carotenoids (fat needed in the meal)
- For some nutrients i.e., proteins, bioavailability is assumed to be additive

Zinc bioavailability	Phytate content - diet characteristics	DRV adult women (European Food Safety Authority, EFSA)
44%	300 mg phytate, refined diet	8
35%	600 mg phytate, semi-refined diet	9.6
30%	900 mg phytate, semi-unrefined diet	11.1
26%	1200 mg phytate, unrefined diet	12.6

Bioavailability of meal/diet should be ultimately considered



Bioavailability of high iron beans is negated when consumed with rice and enhanced when consumed with potato

Figure 1. Experiment 2. Iron uptake as measured by Caco-2 cell ferritin formation from beans pre-treated with phytase. Values represent Fe uptake from normal or high Fe beans with normal phytate (PA; 100%) levels, or PA levels reduced to low (34%) or mid (68%) of the normal PA content. Bar values within food combination groups with no letters in common are significantly different ($p < 0.05$). A single asterisk indicates significant inhibitory effect ($p < 0.05$) of the addition of rice, whereas the double asterisk indicates significant promotional effect of the addition of potato.

Glahn et al. 2017. Iron bioavailability studies of the first generation of iron-biofortified beans released in Rwanda. Nutrients; 9(7)

Bioavailability vs. bioactivity

- Important to consider that different chemical forms of the same nutrient do not necessarily have equal physiological activity
- Vitamin D: D_3 > D_2
  - Vitamin D_3 (cholecalciferol) is more potent and of higher bioactivity than vitamin D_2 (ergocalciferol)
  - D_3 leads to greater increase in serum 25(OH)D and has longer half-life
- Provitamin A carotenoids have lower activity than retinol
  - Plant carotenoids must be converted to retinol in the body
  - Conversion is variable and inefficient in some matrixes
  - Vitamin A equivalency of β-carotene varies: 9:1 – 28:1, 12:1 often used for mixed diets

Take home messages

Biofortification strategies should maximise **nutrient utilisation** by accounting for nutrient stability and bioavailability:

- Breeding for **high bioavailability** – critical for micronutrients like iron
- Targeting of nutrient deposition in edible tissues esp. cereals where outer parts are removed during milling
- Modelling **processing and storage losses** when setting breeding targets
- Several **processing strategies** can be used to improve bioavailability – need to consider context and ease of consistent application
- Ultimately, biofortified crops must be optimised for the real diets into which they will be consumed – ensure **final diet supports absorption**!

Thank you!

Nutritional Composition and Digestibility Lab

We evaluate food sources to develop the most suitable protein and mineral profiles for human food and animal feed.

Our Nutritional Composition and Digestibility Lab (NCDL) is the University of Nottingham's state of the art nutritional analysis facility to help researchers assess nutritional quality of diverse protein sources with a range of cutting-edge techniques.

We house the University's high-throughput *in vitro* protein digestibility platform based on the INFOGEST human *in vitro* digestion model. We also work closely with the elemental analysis team at he University to look at mineral content and bioavailability.

Contact us >

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