Effects of legume peptides formed during *in vitro* digestion on the bioaccessibility of fortified Fe and Zn

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- Dietary Fe and Zn deficiencies contribute to severe health outcomes affecting 2 billion people globally[1].
- Legumes are widely consumed food staples, yet the effects of their proteins on the bioaccessibility of mineral salts, as commonly fortified form of Fe/Zn, remain unclear[2].
- Legume proteins possess unique qualities that may reduce or promote mineral solubility.
- We examined the bioaccessibility of Fe^{3+}/Fe^{2+} and Zn salts with and without protein extracts of pea, lupin and faba using *an in vitro* gastrointestinal digestion model.

Materials and Methods

Water- and NaCl-soluble proteins of defatted pea, faba and lupin (referred to as albumin and globulin), were extracted, filtered and isoelectrically precipitated. Anti-nutritional analyses (phytate & total soluble phenolics) were performed on these crude protein extracts.

The proteins were then solubilised in HEPES buffer, mixed with 20 mM solutions of $FeCl_2$, $FeCl_3$ or $ZnCl_2$, and subjected to *in vitro* gastrointestinal digestion involving oral, gastric and small intestinal fluids. Both fasted and fed conditions were examined for small intestinal digestion, that differed in transit time (2 or 3 h) and bile concentration (0.5 or 30 mg/mL of digestion fluid).

The soluble components of the digesta were analysed for total Fe/Zn (ICP-OES), soluble protein (BCA Assay), and degree of hydrolysis (OPA reagent assay).

Both one-way and multivariate ANOVA were performed for each element with Dunnett's post-hoc vs. the control mineral salt, to analyse the mean differences and source of variation.

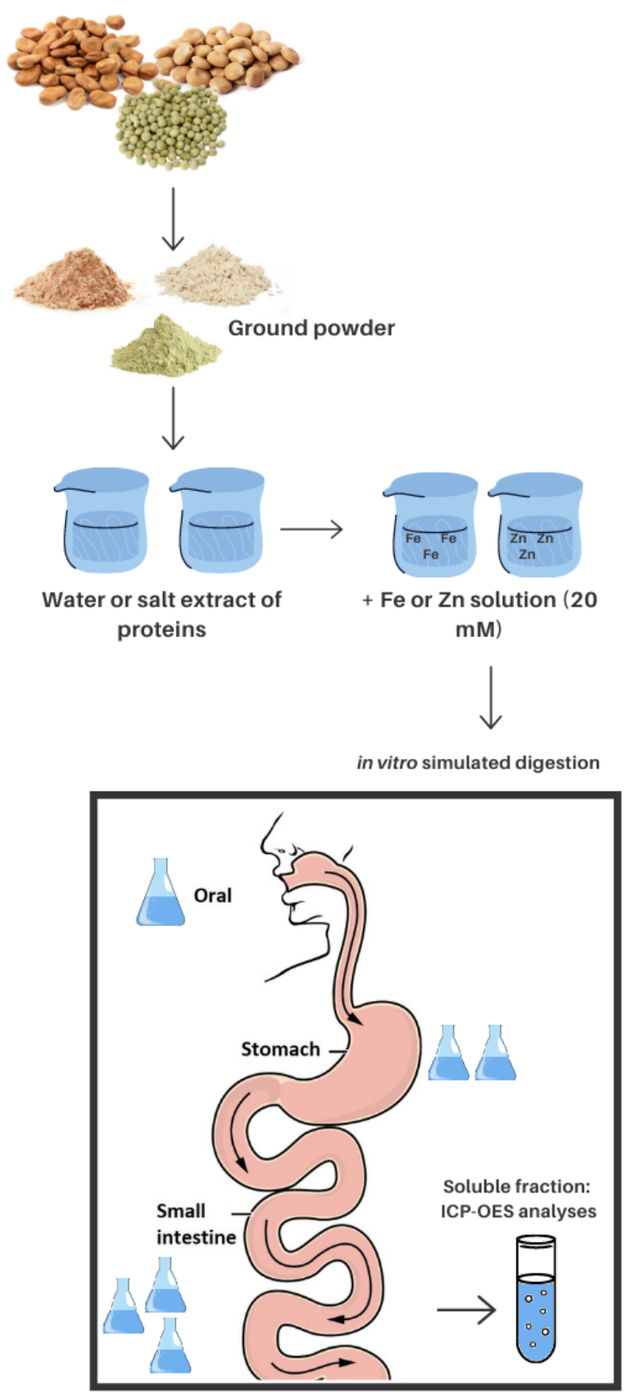

Figure 1: A simplified schematic of the study involving protein extraction and *in vitro human* gastrointestinal digestion.

Results and Discussion

Effects from legume protein dependent on digestion state

- Amounts of soluble minerals by the end of small intestinal digestion are the most important, as it is the major absorption site for both Fe and Zn.
- Following the fasted intestinal state, most peptide fractions had no effect or significantly lowered Fe and Zn solubility. The exception was faba globulin, which enhanced Fe^{2+} and Zn solubility compared to control salts without legume proteins.
- Under the fed intestinal state, the presence of all globulins (from pea, faba or lupin) enhanced Fe^{2+} solubility versus the control without legume proteins. Lupin globulin also enhanced Fe^{3+} solubility versus the control (**Figure 2**).
- Significantly higher levels of total solubilised protein were found in globulins during fed intestinal digestion compared to fasted intestinal digestion, indicating that salt-soluble proteins released during fed digestion play a role in enhancing Fe solubility. However, the relationship between total soluble protein and Fe/Zn bioaccessibility was not consistently linear.

Phytate a likely contributor to lower Fe/Zn bioaccessibility

- The isolated protein fractions were low in soluble polyphenols (<2 mg gallic acid equivalent per gram solids), but contained phytate (193 µmol in lupin globulin, and 1.9 mmol in pea albumin).
- The elemental salts in molar excess of PA may have contributed to some effects of low Fe and Zn solubility during intestinal digestion.

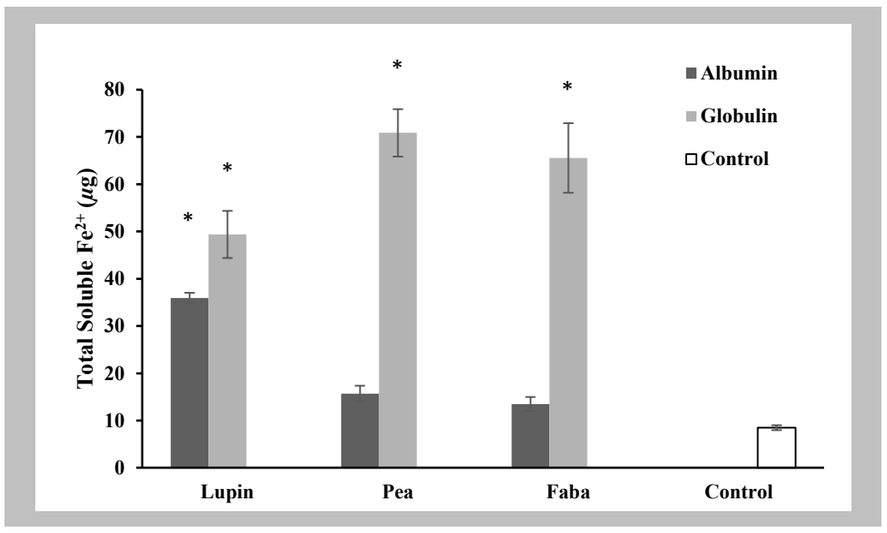

Figure 2: Soluble Ferrous (Fe^{2+}) from mineral salt control and mineral fortified legume protein fractions following sequential oral-gastric-fed intestinal (GI-Fed) digestion. Values with an asterisk (*) are statistically significantly different ($P < 0.05$) from that of the mineral salt control in white. Results are expressed as means ± SEM (n = 3).

Conclusion

We found varying effects of legume protein fractions on Fe and Zn bioaccessibility, based on the digestion stage, Fe oxidation state, and type of protein.

Most albumins were ineffective at promoting mineral solubility during intestinal digestion, particularly the fasted stage. However, legume globulins were able to promote Fe^{2+} solubility, as well as Fe^{3+} for lupin globulin during fed digestion. Zn bioaccessibility during the fed stage was also enhanced by lupin albumin, but not influenced or decreased by other protein fractions.

Promotive effects are likely related to the type of proteins solubilised during this stage that formed soluble complexes with Fe/Zn, but this requires further elucidation.

[1] Tulchinsky, T. H. (2010). "Micronutrient deficiency conditions: global health issues." Public health reviews **32**(1): 243-255.
[2] Zhang, Y. Y., et al. (2020). "Revisiting phytate-element interactions: implications for iron, zinc and calcium bioavailability, with emphasis on legumes." Critical Reviews in Food Science and Nutrition: 1-17.

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