

# 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 ions

Elien Lemmens<sup>1</sup>, Niels De Brier<sup>1</sup>, Kathryn Spiers<sup>2</sup>, Peter Goos<sup>3</sup>, Erik Smolders<sup>4</sup> and Jan A. Delcour<sup>1</sup>

<sup>1</sup>Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LForCe), KU Leuven, Belgium

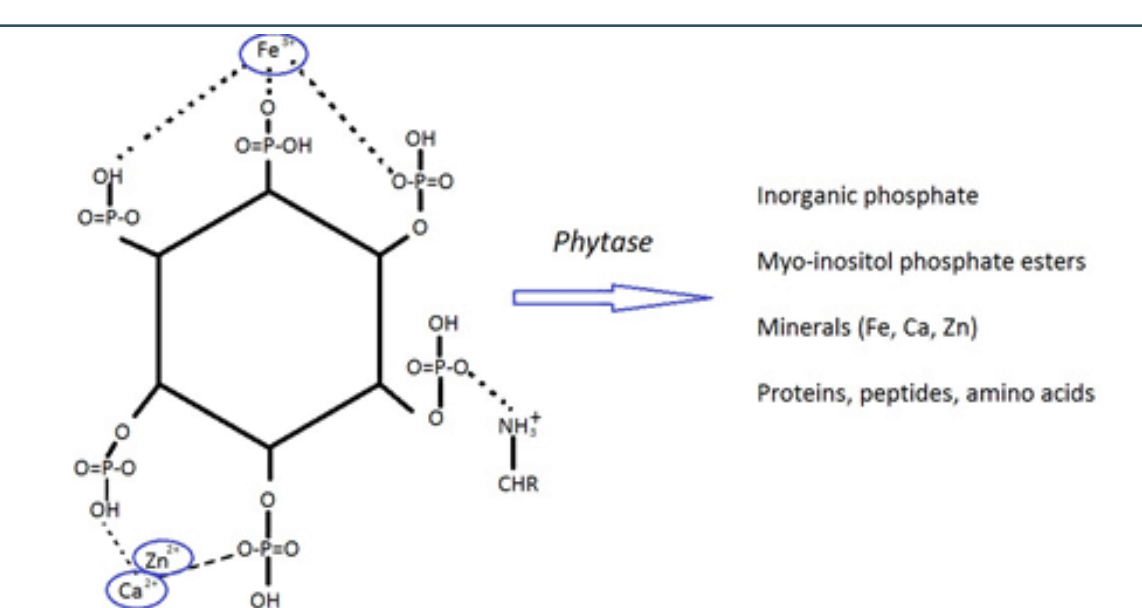
<sup>2</sup>Photon Science, Deutsches-Elektronen Synchrotron DESY, Hamburg, Germany

<sup>3</sup>Division of Mechatronics, Biostatistics and Sensors (MeBioS), KU Leuven, Belgium

<sup>4</sup>Division of Soil and Water Management, KU Leuven, Belgium

## Introduction

(Di)valent minerals such as iron (Fe) and zinc (Zn) occur as phytate chelates in wheat grains which are only poorly digested or absorbed by the human body [1]. The elemental bio-accessibility hence amounts only 5 to 10% [2]. Under certain dietary circumstances, this can lead to Fe and Zn deficiencies [3]. During seed germination, hydrolytic enzymes are synthesized and secreted to mobilize grain reserves that fuel seedling growth and development [4]. We hypothesize that the increase in phytase activity during germination makes phosphate, mineral elements and myo-inositol available for plant growth and development and, when used in food systems, for human uptake.



## Approach



## Experimental

### Multifactorial design of experiments

We evaluated the impact of 19 different factor level combinations on phytate hydrolysis in (germinated) wheat. During (germinated) wheat hydrothermal processing the incubation time ranged from 2 to 24 h, the incubation temperature from 20 to 80 °C and the pH from 2.0 to 8.0.

### Elemental mapping

Elemental  $\mu$ -XRF maps were collected at the Petra III synchrotron facilities in Hamburg, Germany. Element maps of 85  $\mu$ m thick cross sections of the middle part of the grain were collected [5]. A beam energy of 10 keV was used and the fluorescent signal was collected using a 384-element Maia detector system.

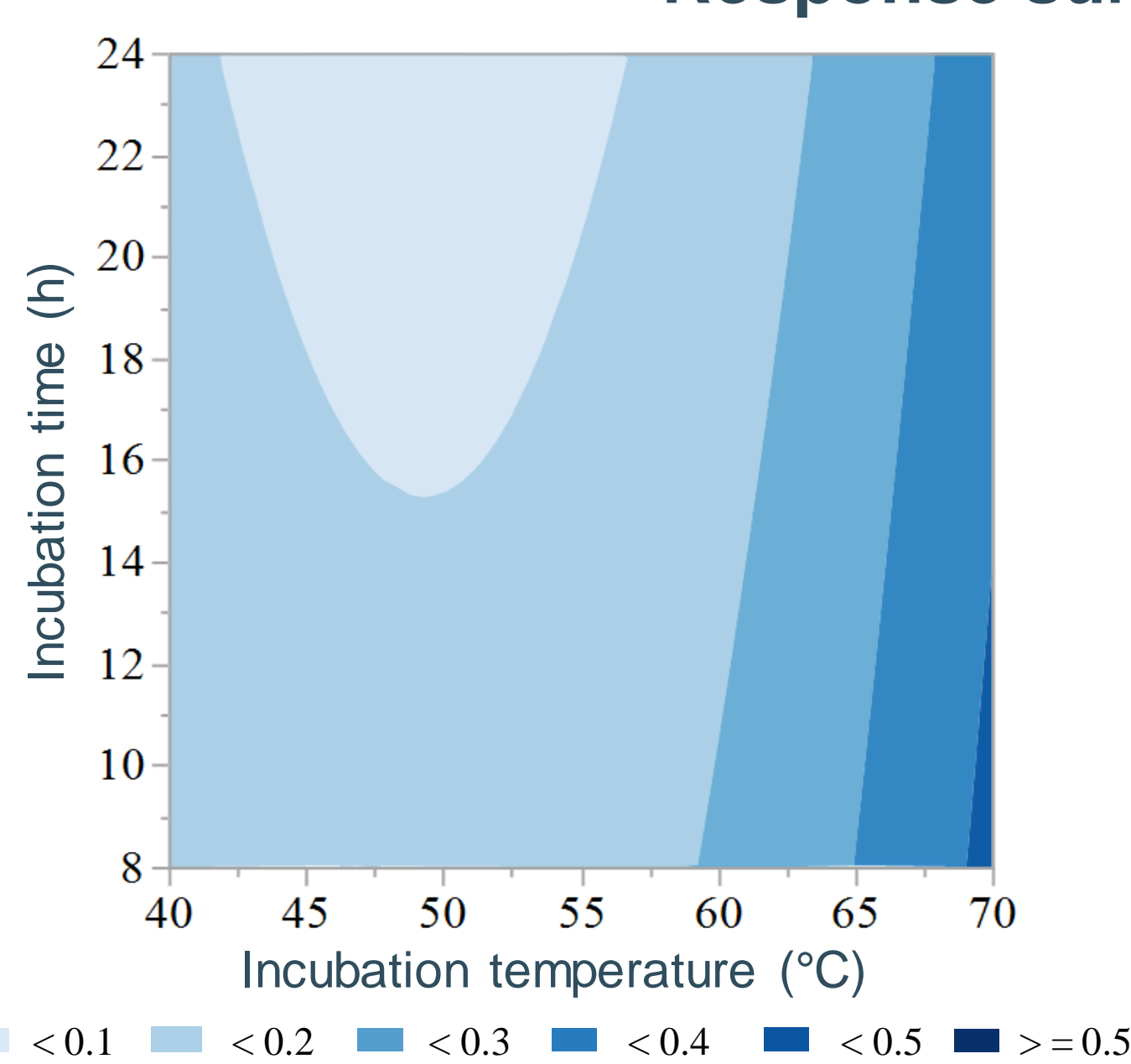
### Fe X-ray absorption edge structure (XANES) imaging

XANES imaging consisted of 'stacks' of  $\mu$ -XRF maps obtained by scanning the entire area of interest 74 times while progressively increasing the energy from 7076 to 7244 eV across the Fe K-absorption edge [5].

### In vitro mineral bio-accessibility

An *in vitro* digestion procedure was carried out in which the digestion by mouth (37 °C, pH 7.2), stomach (37 °C, pH 2.0, pepsin) and small intestine (37 °C, pH 6.8, pancreatin) were simulated. The obtained suspension was then centrifuged and the elemental concentrations in the digested fraction were determined using inductively coupled plasma mass spectrometry.

## Response surface model



The statistical model ( $R^2 = 93\%$ ) predicted that hydrothermal processing of wheat germinated for 120 h at 50 °C and pH 3.8 for 24 h degraded the phytate structures by 95%.

Fig. 1. Predicted phytate content (% initial dry matter) in a 120 h germinated wheat grain at pH 3.8

## Fe speciation

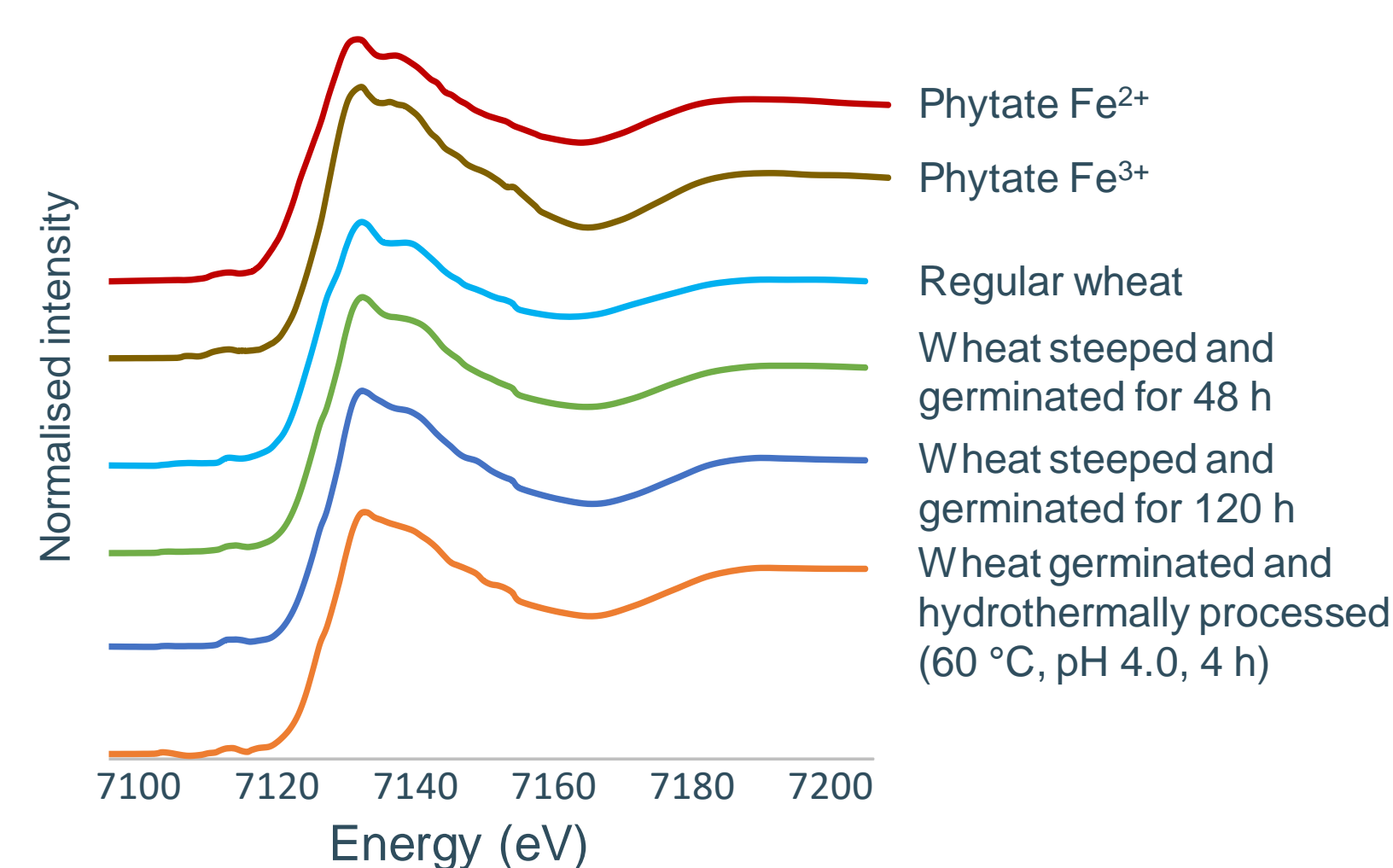


Fig. 3. Fe K-edge XANES spectra

Fe bound to phytate exhibits a typical shoulder feature at around 7137 eV [6] which was also present in the spectra of aleurone cells of regular wheat, clearly exhibited other features at the Fe K-edge, indicating that other Fe species occurred in these samples and that Fe was (partly) released from phytate chelates upon processing.

## Mineral distribution

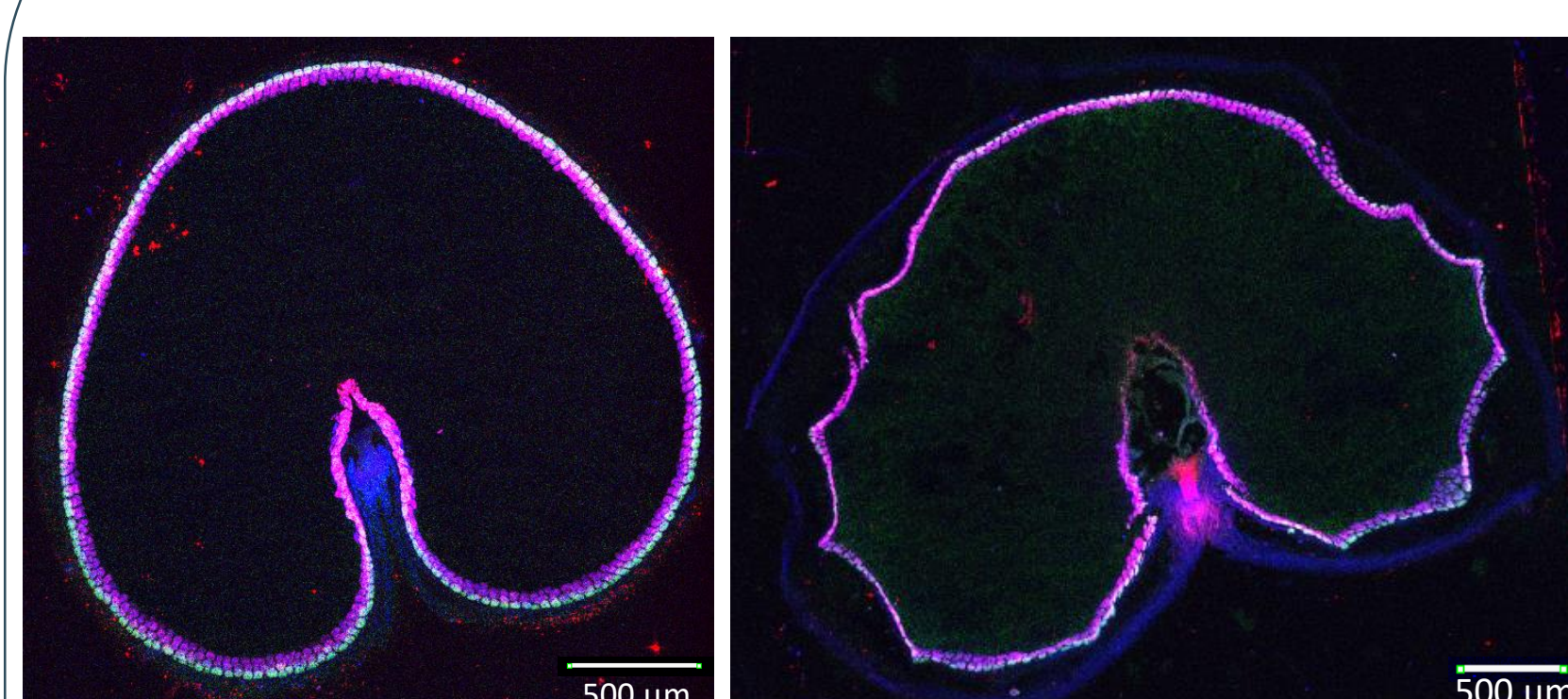


Fig. 2. Elemental maps of cross sections of a regular wheat grain (left) and a wheat grain germinated for 48 h and hydrothermally processed at 60 °C, pH 4.0 for 4 h (right)

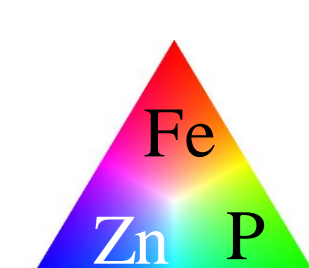


Fig. 2. shows that Fe and Zn were mainly present in the aleurone in wheat where they co-located with P suggesting that they were chelated to phytic acid. Germination and hydrothermal processing did not affect the localization of Fe while Zn was also detected in the pericarp after processing. One can assume that Zn partly migrated from the aleurone to the pericarp once it was released from the phytate structures.

## Mineral bio-accessibility

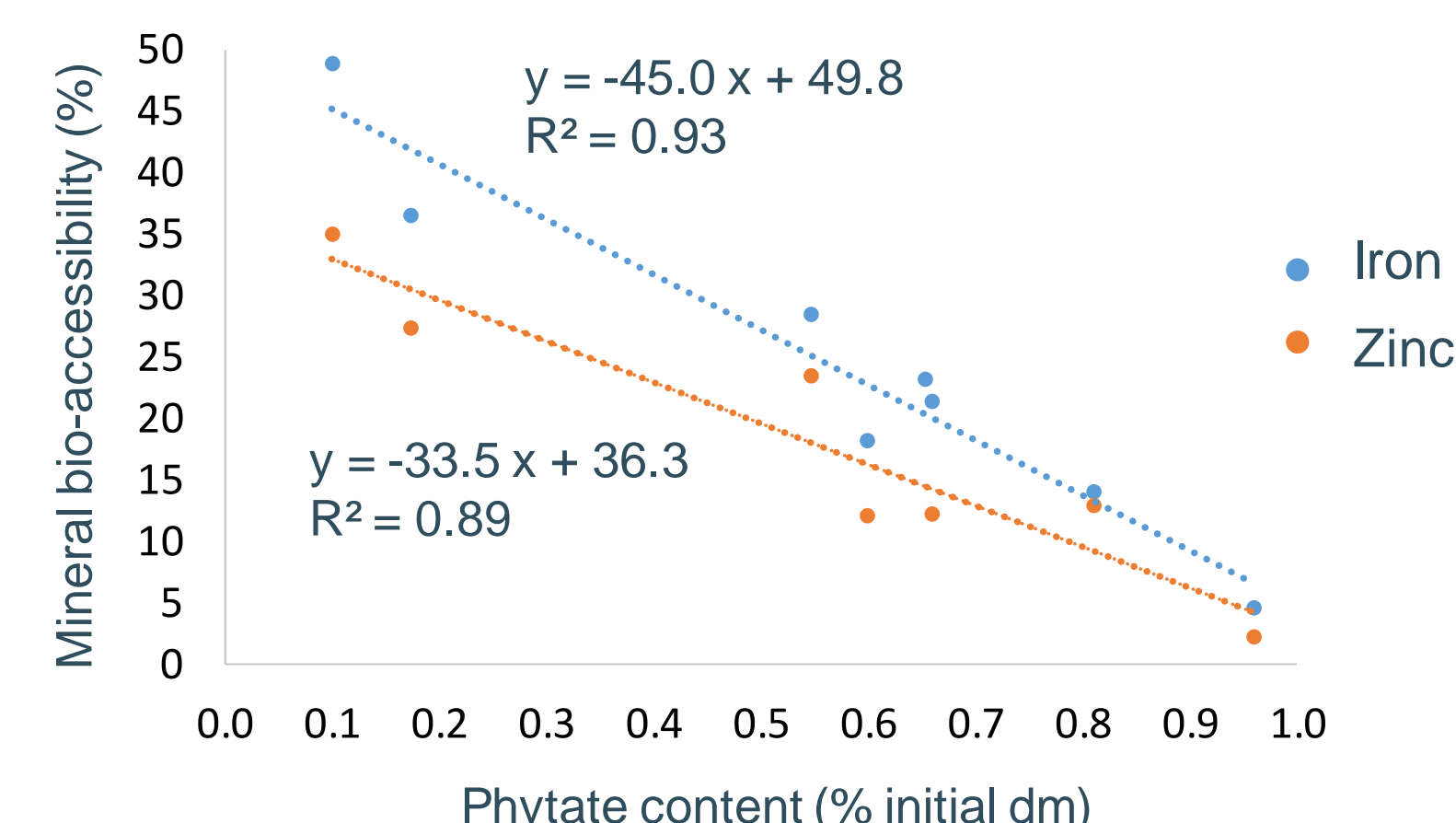


Fig. 4. Mineral bio-accessibility as a function of phytate content in (processed) wheat grains

Hydrothermal processing of 120 h germinated wheat grains at 50 °C, pH 3.8 for 24 h increased **Fe bio-accessibility from about 5 to 50% and Zn bio-accessibility from about 3 to 35%**. Fig. 4. shows that there was a strong linear correlation between the phytate content in (processed) wheat grains and the bio-accessibility of both Fe and Zn.

## Conclusions

Hydrothermal processing of germinated wheat using conditions allowing optimal phytase action (50 °C, pH 3.8, 24 h) led to an almost complete phytate breakdown. Furthermore, Fe was not translocated during processing, while Zn (partly) migrated to the pericarp. The chemical speciation of Fe ions in wheat grains drastically changed upon hydrothermal processing, indicating that they were no longer bound to phytate structures. The extensive phytate breakdown in these samples increased Fe bio-accessibility from about 5 to 50% and Zn bio-accessibility from about 3 to 35%. These innovative process strategies can pave the way for increasing the content of bio-accessible mineral elements in whole grain-based products.

## References

[1] Sandberg, A. & Andersson, H. (1988), The Journal of Nutrition 118, 469-473. [2] Lemmens, E. *et al.* (2018), Food Chemistry 264, 367-376. [3] Beal, T. *et al.* (2017), Plos one 12, 1-20. [4] Bewley, J.D. *et al.* (2013), Springer, New York, USA. [5] De Brier, N. *et al.* (2016), Plant, Cell and Environment 39, 1835-1847.