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Società Italiana di Agronomia
XLV Convegno Nazionale

Sassari 20-22 settembre 2016

Agronomic biofortification affects iron and zinc concentration and nutraceuticals in wheat flour and bread

Valentina Ciccolini, **Antonio Coccina**, Elisa Pellegrino, Laura Ercoli

Sassari, 22th September 2016

Micronutrient deficiencies of iron and zinc affect more than 2 billion of people worldwide

(WHO, 2012)

Fe

Iron deficiency causes anaemia, reduced effort and resistance to infections bone health (Fardet, 2010)

Zn

Zinc deficiency causes various health complication such as growth stunting, impaired mental development and pneumonia (Fardet, 2010)

In countries with high incidence of micronutrient deficiencies, cereal based food represents the largest proportion of the daily diet

(Cakmak et al., 2010)

Cakmak et al. (2010) *Cereal Chem* 87, 10; Fardet (2010) *Nutr Res Rev 23*, 65;WHO (2012) *The World Health Report*.

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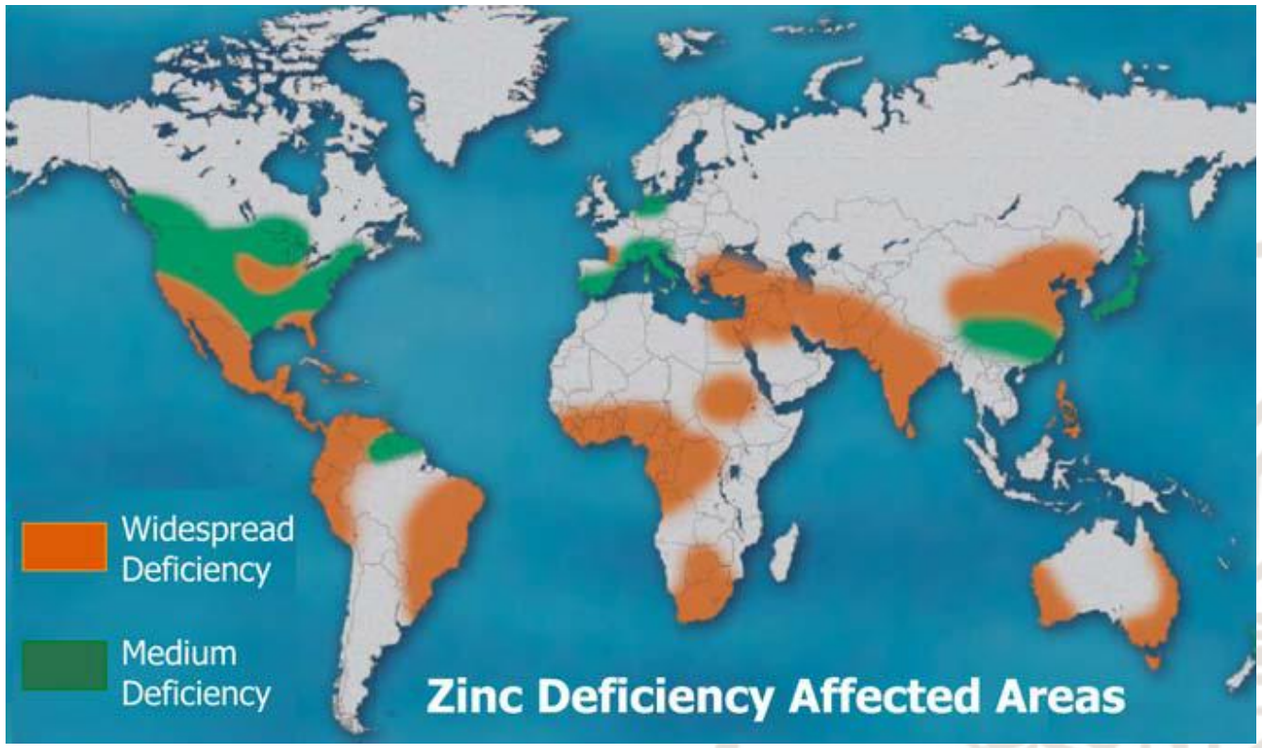
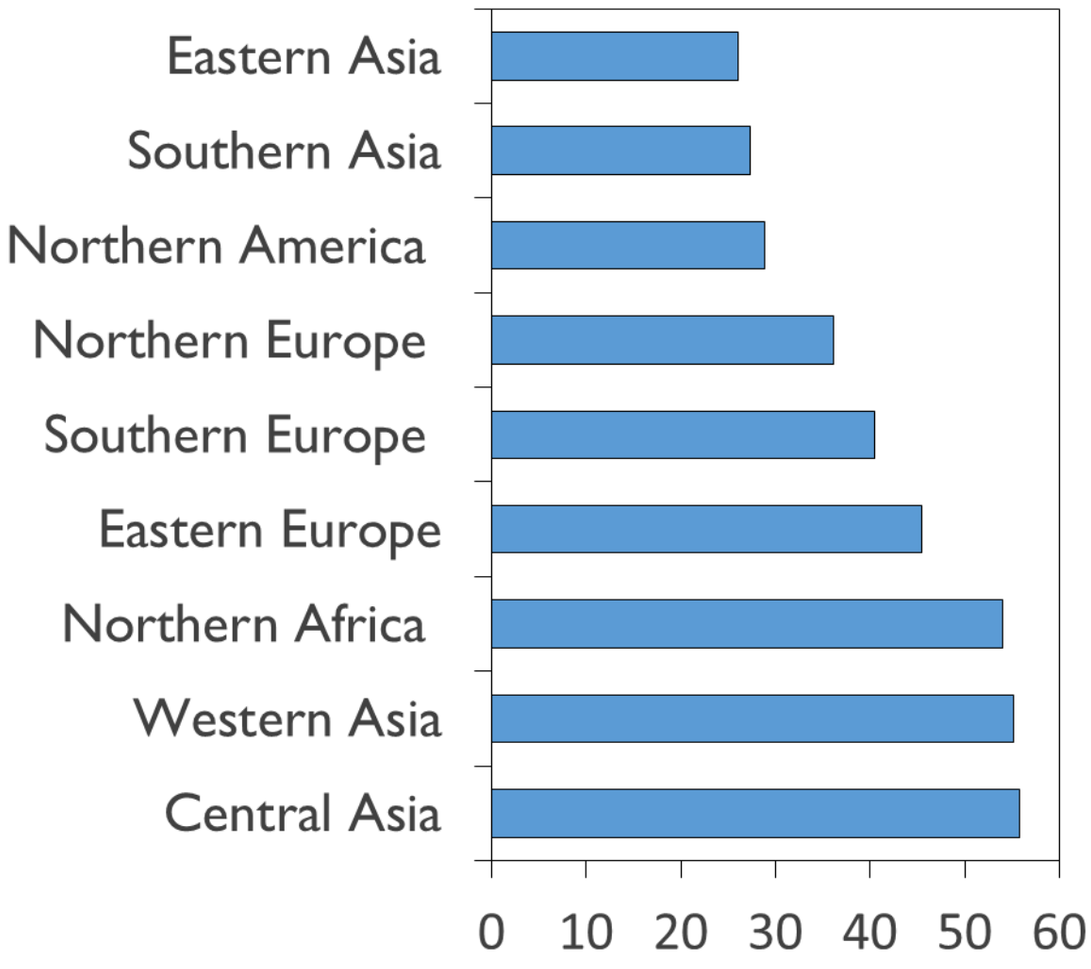
Areas of soil Zn deficiencies



From: Cakmak (2008) *Plant Soil* 302, 1

Daily calory intake from wheat in world regions (%)



From: http://faostat3.fao.org
FAO Database, 2011

Agronomic biofortification of staple food is an approach to tackling micronutrients deficiencies

Biofortification is a process to improve micronutrient concentrations in edible parts of the crop plants. Three strategies are available:

1. **Conventional plant breeding** → by breeding crops with an increased ability to accumulate micronutrients
2. **Transgenic approachs** → by targeting genes that increase micronutrients content in edible parts or reduce the concentration of antinutrients
3. **Agronomic biofortification** → by soil or foliar fertilization of crops with micronutrients

Application method

Fertilizer chemical form

Rates & Splitting

Growth stages

Cakmak (2008) *Plant Soil* 302; White and Broadley (2009) *New Phytol*, 182, 49

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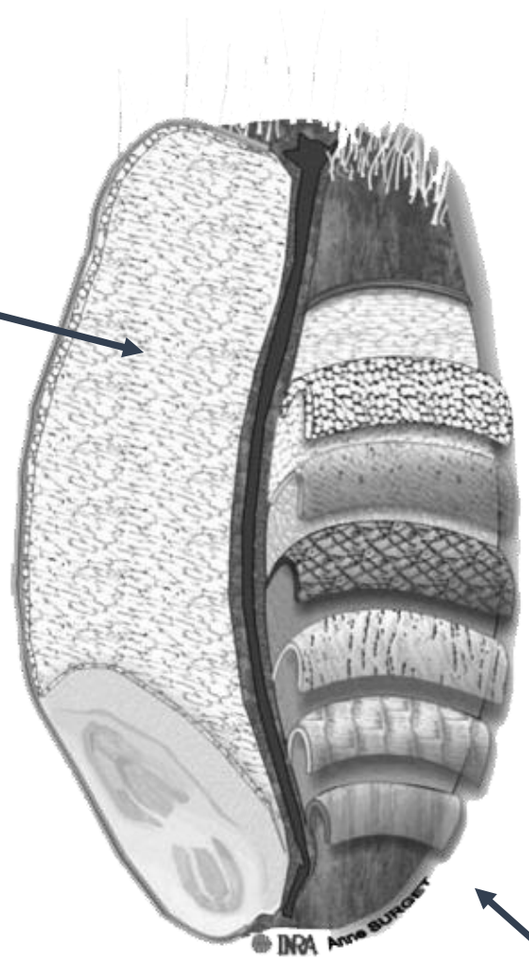
Micronutrients: where in grain?

Starchy endosperm

- Starch and proteins
- Carotenoids
- Thiamin (B1)
- Selenium

Aleurone layer

- Antioxidants (poliphenols, flavonoids)
- Vitamin E
- B vitamins
- **Minerals (Fe and Zn)**
- Phytates
- Enzymes
- Alpha-lipoic acid
- Beta-glucans
- Soluble and insoluble dietary fibre



Germ

- Lipids
- Flavonoids
- **Minerals (Fe and Zn)**
- Vitamin B and E

From: Fardet (2010) *Nutr Res Rev 23*, 65

Aims

Does agronomic biofortification improve grain yield and Fe and Zn concentrations and nutraceuticals in whole-wheat flour?

Does the response to biofortification vary between wheat varieties?

Does bread making affect the concentrations of Fe and Zn and nutraceuticals?

Experimental site

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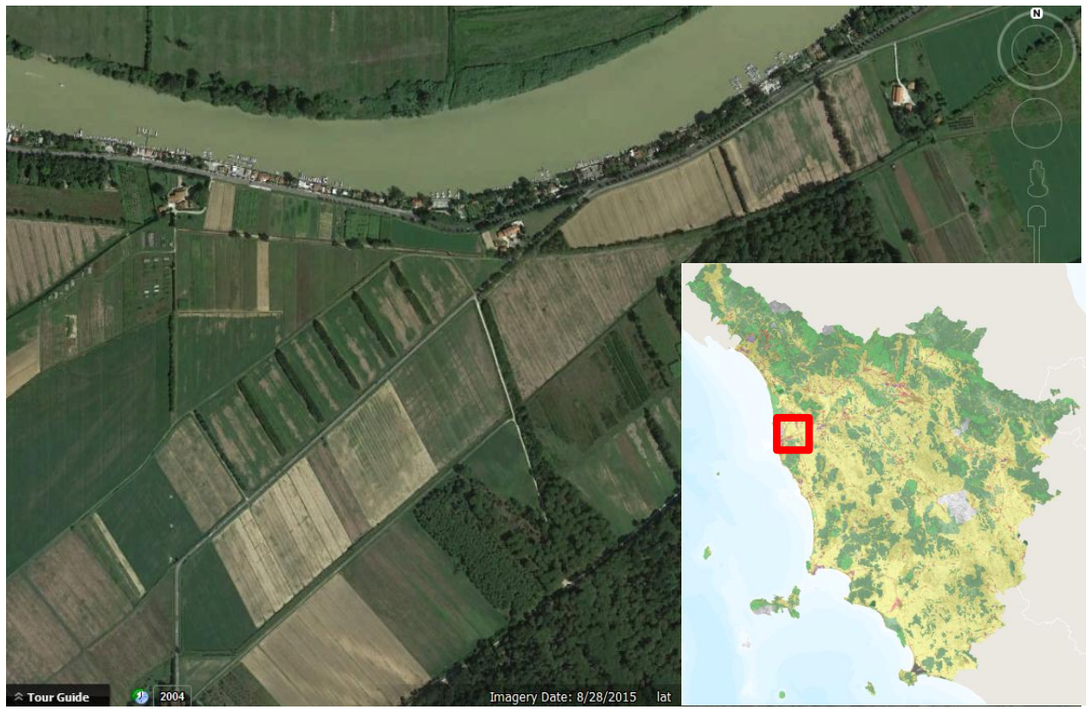


Enrico Avanzi Interdepartmental Centre for Agro-Environmental Research (CIRAA) of the University of Pisa - San Piero a Grado (Pisa)
43°40′ N, 10°21′ E

Mediterranean climate
Precipitation → 880.3 mm year^{-1}
Minimum temperature → 10.2 °C
Maximum temperature → 20.0 °C

Soil texture → silty-clay

Soil available Fe and Zn concentrations
(DTPA soil test; Lindsay & Norwell, 1978)

Fe → 21 mg kg^{-1} Medium availability

Zn → 0.48 mg kg^{-1} Low

Experimental design

Experimental design

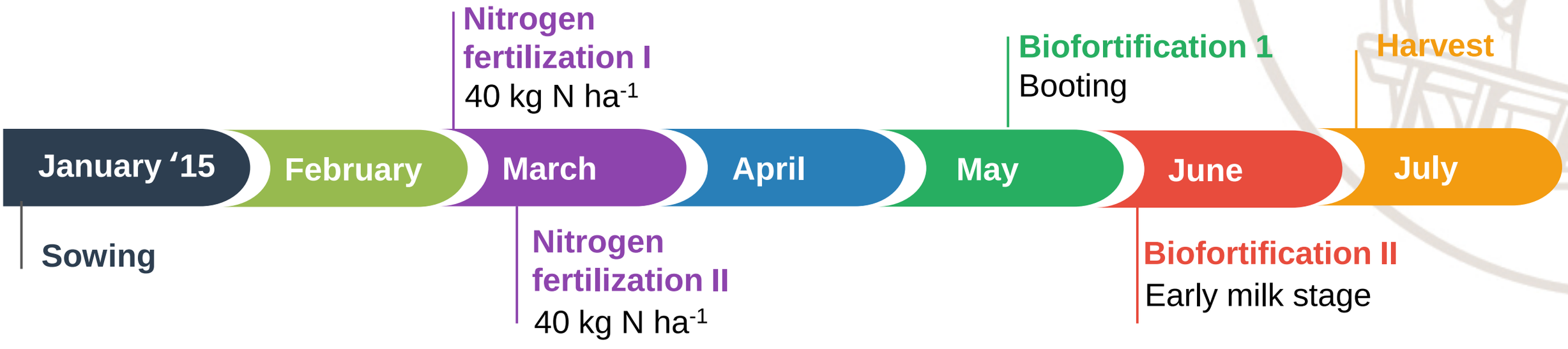
- Full factorial experiment arranged in a completely randomized design with 3 replicate plots (15 m^2)

Factor 1: Two bread wheat varieties (*Triticum aestivum* L.)

- Gentil Rosso (Old) *vs* Blasco (Modern)

Factor 2: Agronomic biofortification with Iron (Fe) and Zinc (Zn)

- Foliar application of 0.19% (w/v) FeSO4 and ZnSO4 → 3.80 kg ha^{-1}
  The solution was supplied twice at the rate of 100 mL m^{-2}



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Milling…

Whole-wheat flour

…and bread making

Whole wheat flour (Old and Modern): 40%
Water: 40%
Sourdough: 20%
Total baking time: 55 min (at 220 °C)

Chemical analyses of bread and flour

Iron and Zinc

Fe and Zn concentration was determined by atomic absorption spectroscopy (Isaac et al., 1998)

Total polyphenol and flavonoid concentration

Extraction: 1g of sample + 10 mL of Methanol 80% (HCl 1N 80:20 v/v). Colorimetric procedure (Adom et al., 2005)

Total antioxidant activity

Extraction: 1g of sample + 10 mL of Methanol 80% (HCl 1N 80:20 v/v) Total antioxidant activity was assessed by 2,2-diphenil-1-picrylhydrazyl radical (DPPH) assay (Kulkarni et al. 2006)

Phytate

Extraction: 60 mg of sample +9 mL HCl 0.2N
Colorimetric procedure (Hussain et al. 2011)

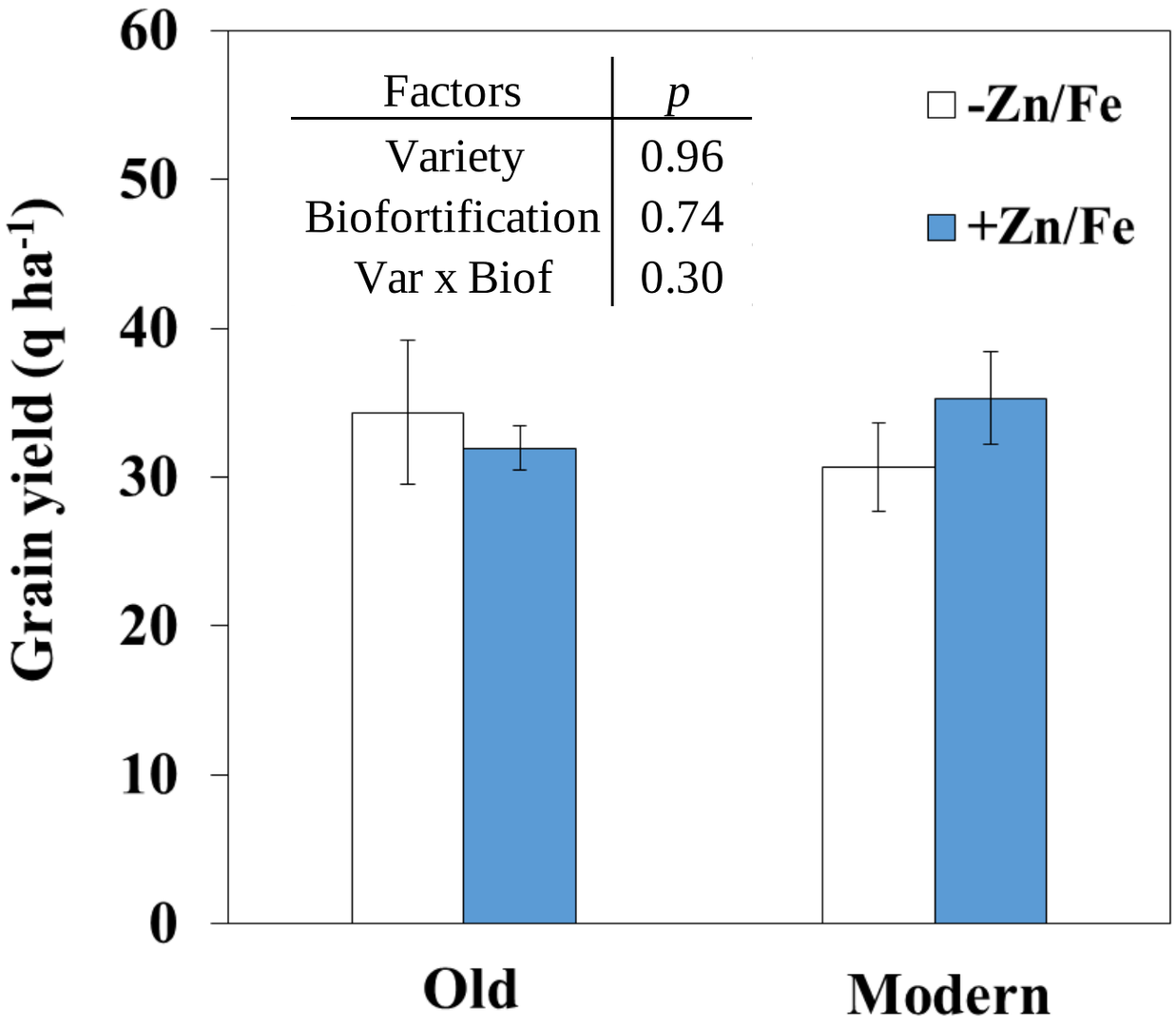
Alpha-lipic acid

ALA concentration was determined by HPLC (Sgherri et al., 2002)

Adom et al. (2005) *J Agricul Food Chem 53*, 2297; Hussain et al.(2011) *Cereal Res Commun* 40, 62; Kulkarni et al. (2006) *Phytoth Res 20*, 218-227; Sgherri et al. (2002) *Plant Physiol Biochem 40*, 591

Results

Does agronomic biofortification improve grain yield?



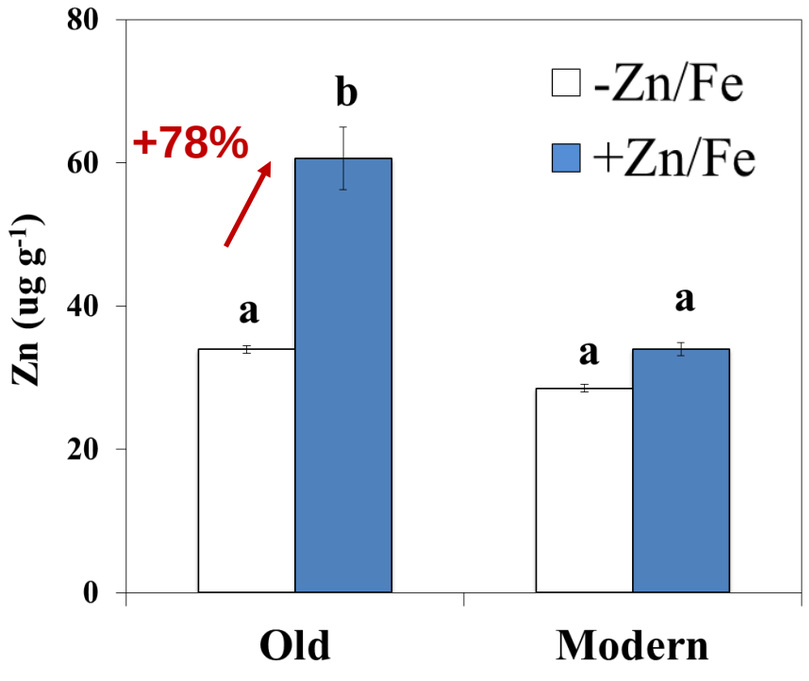
Results

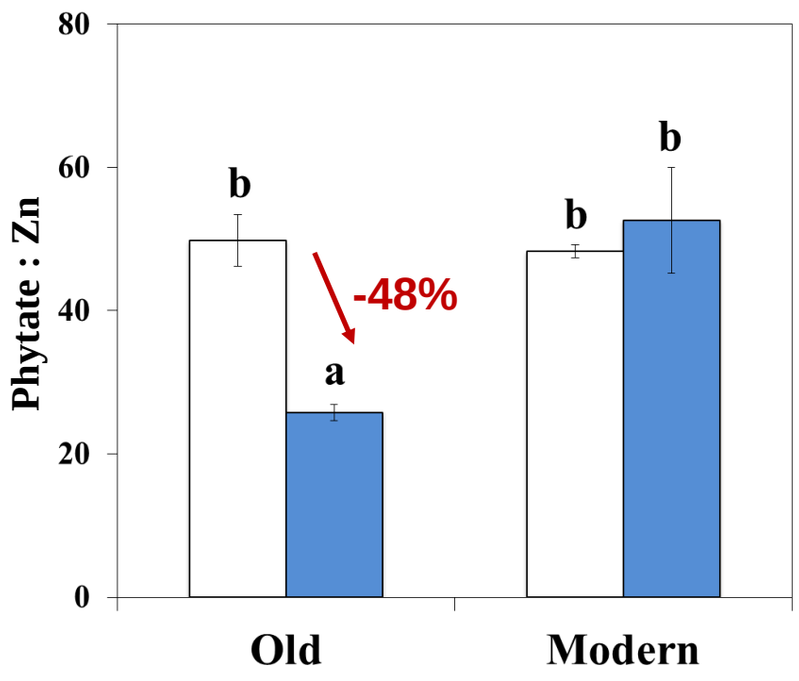
Does agronomic biofortification improve Fe and Zn concentrations and nutraceuticals in whole-wheat flour?

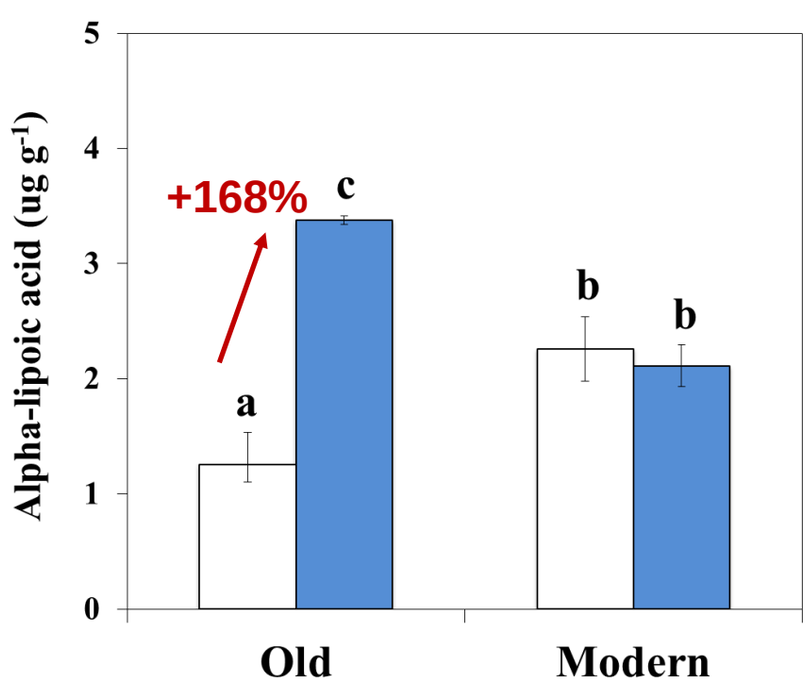
P- value of 2-way ANOVA to evaluate the effect of variety and foliar biofortification with iron and zinc on nutritional and nutraceutical values of bread wheat flour (*Triticum aestivum* L.)

Factors[a]	Fe	Zn	Poliphenols	Flavonoids	Antioxidant Activity	Phytate	Phytate : Fe	Phytate : Zn	Alpha-Lipoic Acid
Variety (Var)[b]	**0.001**	**0.001**	0.248	0.060	**0.038**	0.794	**0.011**	**0.016**	0.495
Biofortification (Biof)	0.417	**0.001**	0.980	0.989	0.343	0.444	0.454	**0.045**	**0.001**
Var x Biof	0.492	**0.001**	0.370	0.957	0.579	0.165	0.059	**0.009**	**0.001**

[a]Var and Biof are used as fixed factors; [b]Bread wheat varieties: Gentil Rosso, Blasco; [c]Bold values are statistically significative ($P < 0.05$).







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Effect of variety on antioxidant activity, Fe concentration and phytate : Fe ratio in whole wheat flour			
Variety	Fe concentration ($\mu g\ g^{-1}$)	Antioxidant Activity (mg AA equivalents)	Phytate : Fe
Old	41.3 b	2.2 b	33.7 a
Modern	29.1 a	1.9 a	46.4 b
	+42%	**+16%**	**-27%**

Results

Does bread making affect the Fe and Zn concentrations and nutraceuticals?

-77% **-71%**

Bakery effects on nutraceuticals and nutritional values of whole wheat flour and bread

Source of variation[a]	Fe concentration ($\mu g\ g^{-1}$)	Zn concentration ($\mu g\ g^{-1}$)	Poliphenols (mg GAE g^{-1})	Flavonoids (mg catechin equiv. g^{-1})	Antioxidant Activity (mg AA equiv. g^{-1})	Phytate (mg g^{-1})	Phytate : Iron	Phytate : Zinc	Lipoic Acid ($\mu g\ g^{-1}$)
Whole wheat flour	35.2 b	39.27 b	6.92	0.82	2.06	16.24 b	40.05 b	44.12 b	2.25 b
Whole wheat bread	29.75 a	35.17 a	7.19	0.31	1.37	3.11 a	9.06 a	9.29 a	0.15 a

[a] Analysis of covariance (ANCOVA): Variety and foliar biofortification are used as covariate.

- 15% **-10%**

WHO thresholds Phytate : Fe/Zn

> 15: low bioavailability
5-15: medium bioavailability
< 5: high bioavailability

-81% **-91%**

Conclusions

- **Biofortification increases Zn, Alpha-lipoic acid concentration and phytate:Zn in whole wheat flour**
- **Old variety responds better to biofortification in term of Zn concentration, phytate:Zn, Fe concentration, and phytate:Fe**
- **Bread making process increases bioavailability of Fe and Zn**

Poster session S4/5

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La ricerca agronomica verso il 2030: gli obiettivi globali di sviluppo sostenibile

Sassari, 20-22 settembre 2016
Dipartimento di Agraria – Università di Sassari

Increase of Iron and Zinc Concentration in Grain of Bread Wheat Field-Inoculated with Arbuscular Mycorrhizal Fungi

Research funded by Assessorato alla Sanità, Regione Toscana

Laura Ercoli, Gaia Piazza, Valentina Ciccolini, Enrico Bonari, Elisa Pellegrino
Istituto di Scienze della Vita, Scuola Superiore Sant'Anna, P.zza Martiri della Libertà 33, 56127 Pisa
Corresponding Author: l.ercoli@sssup.it

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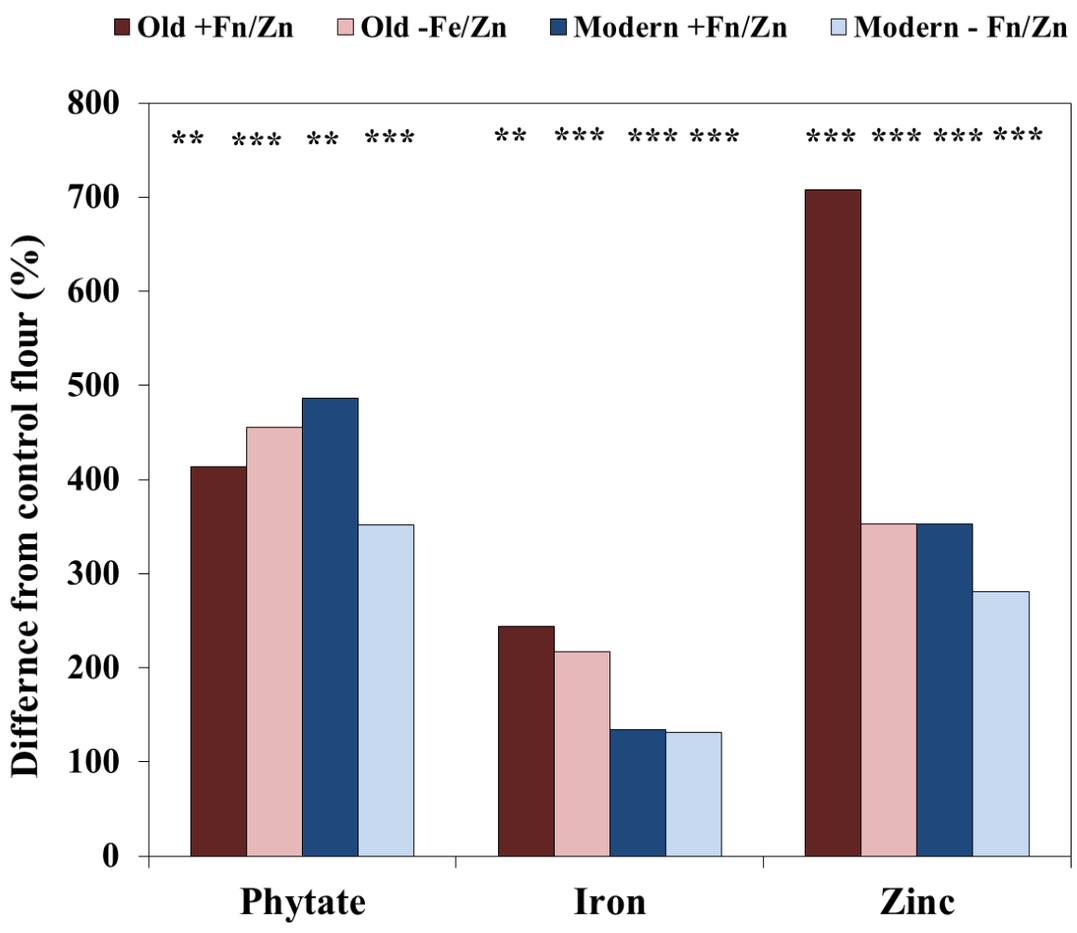
Thanks for your attention!

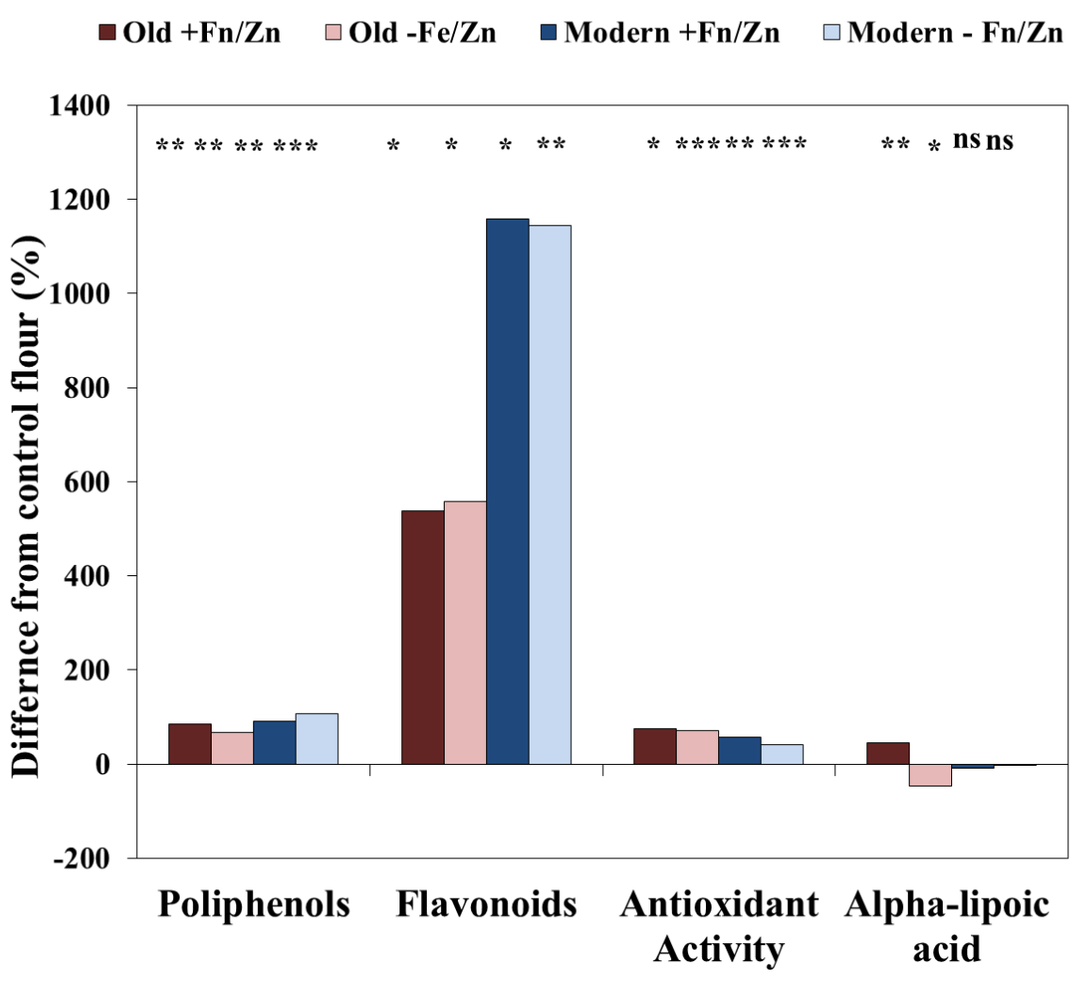
Antonio Coccina
a.coccina@sssup.it

This work is part of the project **FATEPreSco** “Valorizzazione delle proprietà epigenetiche dei genotipi antichi toscani di frumento tenero biofortificati con ferro e zinco nella prevenzione dell’insufficienza cardiaca cronica”

http://www.researchtuscany.com/progetto_dett.asp?lingua=_ita&idlink=3&id_progetto=181

Comparison with control flour





(*t*-test: * $P < 0.05$;** $P < 0.01$;*** $P < 0.001$)

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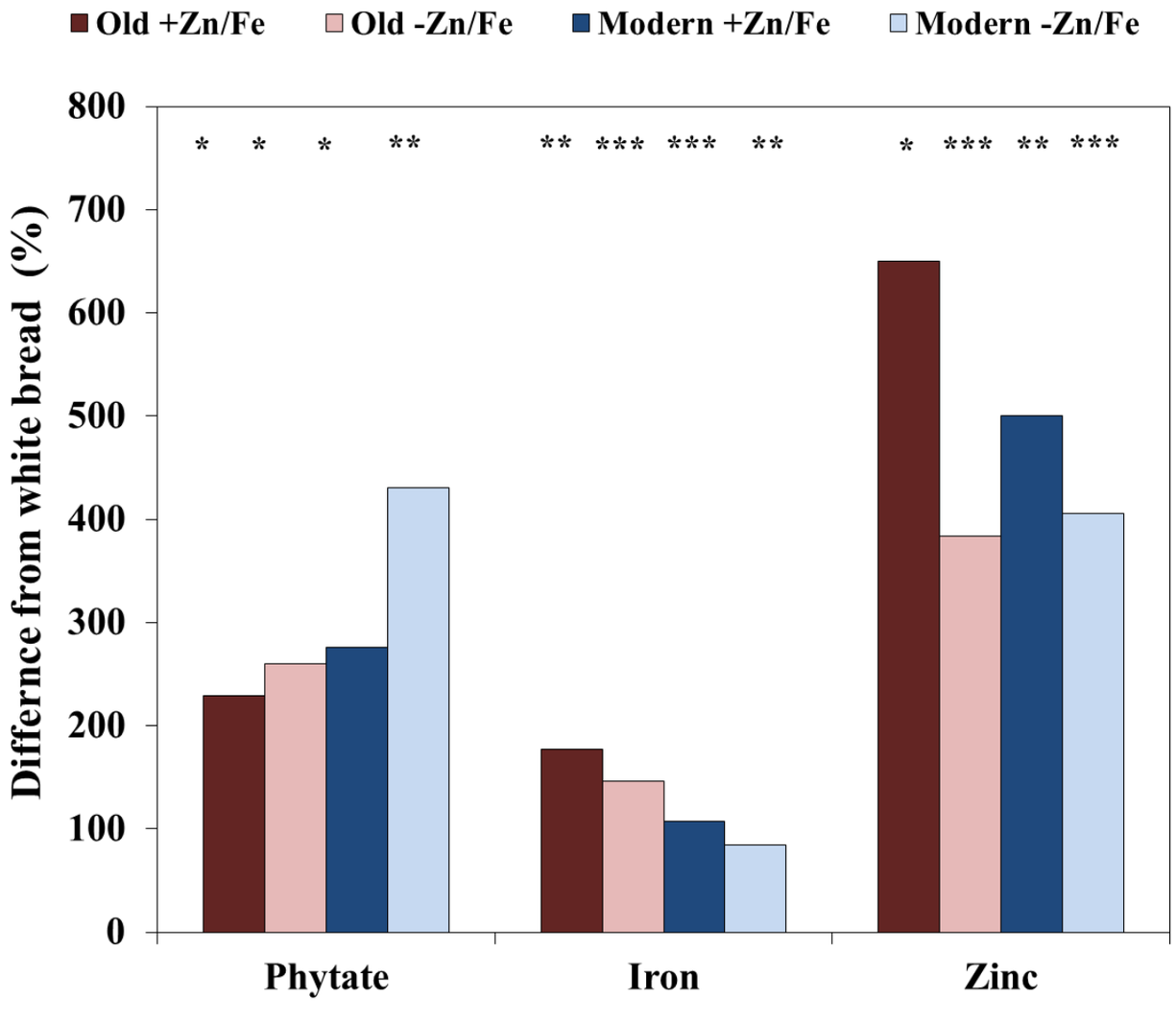
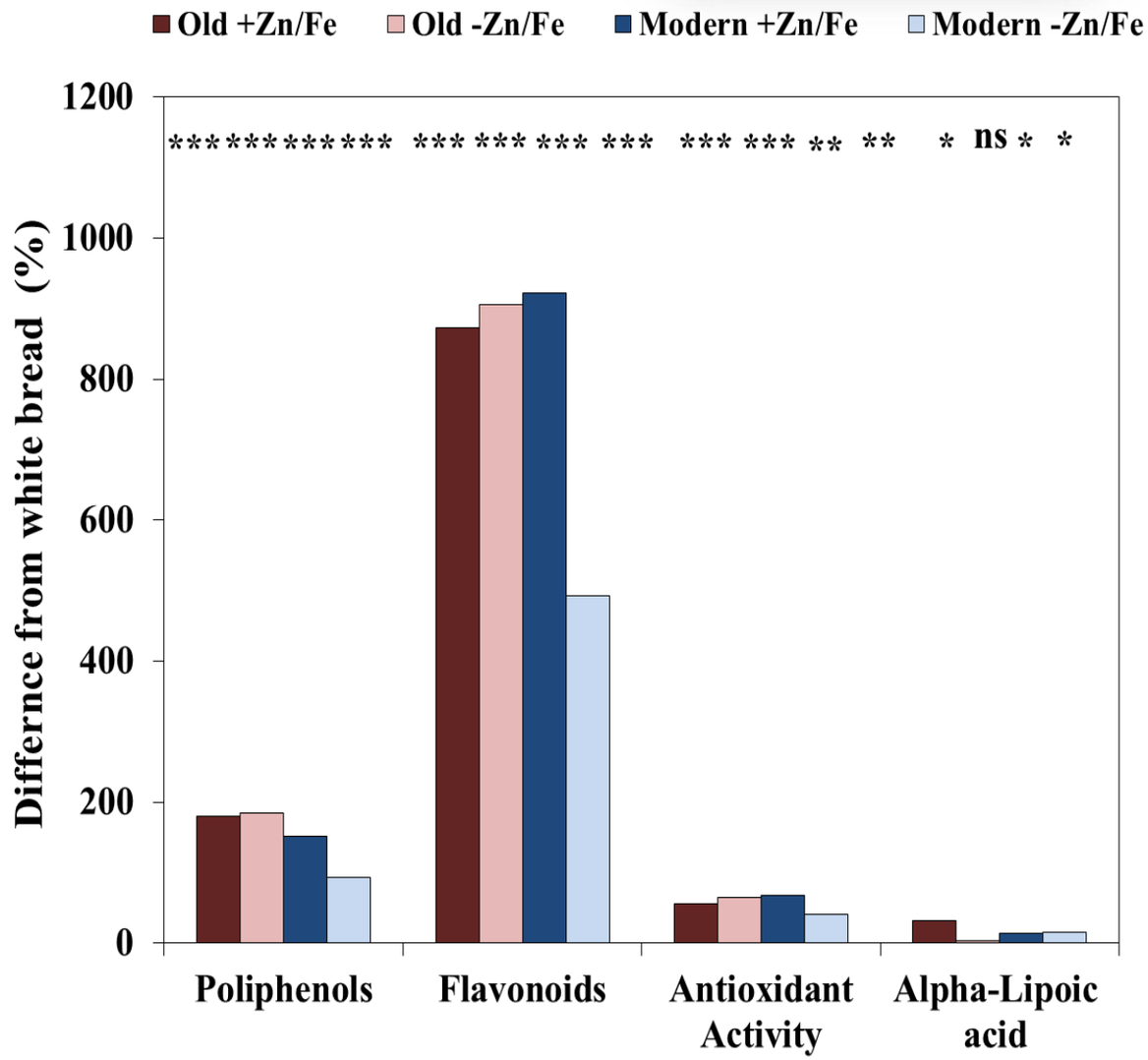
Comparison to white bread





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