The potential of the iron concentrated germinated wheat in wheat flour fortification: an alternative to the conventional expensive iron fortification

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Abstract Investigation of the effect of the Fe abiotic stress on the germination rate, iron accumulation, root and shoot elongation of wheat (*Triticum aestivum*) was carried out. The grains were exposed to different experimental concentrations of ferrous sulfate (FeSO_4) (0–15 mM). The effect of the treatment on the shoot and root elongation of the seeds were reported. There is a linear relationship between the treatment and the inhibition of shoot elongation. The half-inhibition dose (ID_{50}) of FeSO_4 on inhibition of shoot elongation was 7.3 mM. Each treatment groups (3–15 mM) were used to fortify the wheat flour at 0.1 mg Fe/g. The effect of fortification on rheology (farinograph, extensograph, and amylograph), quality of pasta and iron bioavailability was studied. The pasta cooking properties, texture and sensory properties of 12 and 15 mM composite pasta were equally acceptable as wheat without fortification, or NaFeEDTA fortified pasta. The iron dialysability of 3–15 mM composite pasta was similar to the NaFeEDTA fortified pasta. The iron bioavailability (in vivo) of 15 mM group based pasta was evaluated in the anemic rats. The pasta at 4% (Fe 0.026 mg/g) in iron-deficient diet fed to anemic rats for 2 weeks showed percentage iron absorption (PIA) and hemoglobin regeneration

efficiency (HRE) of 85.3% and 44.4% respectively which is higher than the NaFeEDTA. In conclusion, iron-fortified pasta is the promising food fortificant with more iron bioavailability in the prevention of iron deficiency anemia.

Keywords Wheat · Fortification · Iron bioavailability · Anaemic rats · Haemoglobin regeneration efficiency

Abbreviations

HRE	Haemoglobin regeneration efficiency
PIA	Percentage iron absorption
GFE	Gross food efficiency
mM	Millimolar
BU	Brabender units
FU	Farinograph units

Introduction

Iron deficiency anemia occurs when there is an imbalance in the iron intake/absorption and body iron requirement. The predominance of iron deficiency anemia is higher in developing than in the developed countries (Shaw and Liu 2000). Iron deficiency anemia majorly affects children under 5 years, women of childbearing age and pregnant women (Dharmalingam et al. 2010; Ahmad et al. 2010; Buchowski et al. 1989). Dietary diversification, supplementation, and food fortification are widely accepted strategies to combat iron deficiency anemia. Food products successfully fortified with iron include milk, table salt, curry powder, sugar, fish sauce, dairy products, bakery products, maize flour and wheat flour (Sachdeva et al. 2015; Muthayya et al. 2012; Martinez-Navarrete et al. 2002). Among the different food products fortified with iron, wheat flour fortification is a common practice

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worldwide. The universal consumption of wheat bread and low bran content of the wheat flour compared to other cereal flours makes the wheat flour the best choice for iron fortification all over the world (Whittaker and Vanderveen 1990). The wheat products are rich in carbohydrate and protein and low levels/bioavailable essential micronutrients (Cakmak et al. 1996). Ferrous sulfate, ferrous fumarate, and NaFeEDTA are used to fortify the wheat flour. The presence of anti-nutritional factors (phytic acid and polyphenols) in whole wheat flour affects the iron bioavailability of the iron-fortified whole wheat flour (Muthayya et al. 2012). The iron bioavailability from the NaFeEDTA was better than the FeSO_4 fortified foods (Kloots et al. 2004). The use of the NaFeEDTA in wheat flour fortification was limited in the developing countries due to the expensiveness of the fortification. The low bioavailable iron salts were used widely in the fortification programmes in developing countries to overcome the fortification costs (Hurrell et al. 2010). Thus there is a definite need for a novel iron fortificant with improved iron bioavailability to address the iron deficiency in the developing nations. Apart from the inorganic iron fortificants, the iron content in grains can be improved through the genetic engineering or abiotic stress during germination (Kalganekar and Lonnerdal 2009; Lucca et al. 2001, 2002; Zielinska-Dawidziak et al. 2014a; b). The iron concentration in the wheat can be improved through the iron treatment to the germinating wheat. The iron concentration in the germinated grains through the iron-enriched medium (abiotic stress) was previously studied in cereals and legumes especially wheat and soybean (Zielinska-Dawidziak et al. 2014a; b; Zielinska-Dawidziak et al. 2012). The use of the Fe abiotic stress treated germinated wheat as a fortificant in the wheat-based products namely pasta was not reported to date. Thus the effect of fortification of the abiotic stress treated flours in wheat flours on rheology, pasta quality and iron bio-availability were experimented in the present paper.

The present experiment aimed to study (1) The relationship between the iron accumulation in wheat seeds due to Fe abiotic stress and its effect on inhibition of root and shoot length. (2) The effect of abiotic stress treated flour in wheat composite flours on farinograph, extensograph, amylograph and physical and sensory attributes of the pasta. (3) The iron dialysability of the composite pasta in comparison with the NaFeEDTA fortified pasta. (4) The effect of the composite pasta on hemoglobin regeneration efficiency using in vivo anemic rat models.

Materials and methods

Raw materials, chemicals, and reagents

Wheat grains (*Triticum Aestivum*) were procured from the local market, the volume, moisture, ash, protein, falling number, and sedimentation value of the wheat grains were 80 kg/HI, 8.4%, 1.3%, 12.5%, 720 s and 21 ml respectively. The wheat flour was procured from the local market, the moisture, ash, total protein, falling number, sedimentation value, wet and dry gluten percentage of the wheat was 12.2%, 0.45%, 11.3%, 640 s, 23 ml, 30.57%, and 9.81% respectively. Triple distilled water was used throughout the experiment. Pepsin from porcine gastric mucosa (≥ 250 units/mg solid), pancreatin from porcine pancreas ($8 \times$ USP specifications), bile salts (the mixture of sodium cholate and sodium deoxycholate), and dialysis tubing cellulose membrane with a molecular mass cut-off of 14,000 Da were purchased from Sigma. Ferrous sulfate (extra pure analytical grade) and E.D.T.A., ferric monosodium salt (extra pure analytical grade) was purchased from HiMedia Laboratories Limited, Mumbai, India. Hydrochloric acid (37% pure), sodium bicarbonate (extra pure), NaOH (extra pure), and Perchloric acid (70% pure) used were of analytical grade procured from Merck, India.

Abiotic stress treatment for the preparation of sprouts

The iron treatment to sprouting wheat grains was performed according to the method described by Zielinska-Dawidziak et al. 2014b. The wheat grains were soaked in 70% ethanol solution for 15 min and washed in running tap water followed by distilled water. Further, the seeds were soaked for 4 h in FeSO_4 solution at the concentrations 0, 3, 6, 9, 12, and 15 mM. The seeds were allowed to germinate in individual trays for 5 days and sprinkled with the respective FeSO_4 solution once in a day. The inhibition of the shoot and root length of the germinated wheat was determined according to the method explained by Wang and Zhou (2005), the shoot and root length of the germinated wheat was measured, and the percentage inhibition of the shoot and root elongation was calculated in comparison with the germinated wheat without iron treatment. The seeds were dried in a tray drier at 50 ± 5 °C until 14% seed total moisture content was attained and ground to powder in Hammermill (Perten Sweden).

Fortification and analysis of flour quality

The wheat flour was fortified with either NaFeEDTA or abiotic stress treated flours (3 mM to 15 mM) at 0.1 mg

Fe/g was given in Table 1. Fortified wheat flour was analyzed for the falling number, farinograph, and amylograph characteristics according to AACC methods (2000). The dough properties of the fortified wheat flours namely water absorption, dough stability were analyzed using farinograph (Brabender, Germany), coupled to a personal computer with graphical data acquisition (Brabender Farinograph Version: 4.2.3) and graphical data correlation (Brabender Farinograph Data Correlation Version: 4.1.2). The pasting characteristics namely peak viscosity and setback of the fortified wheat flour were evaluated using Micro Visco-Amylo-graph (Brabender, Germany), connected to the computer with graphical data acquisition software (Brabender Viscograph Version: 4.5.0) and graphical data correlation software (Brabender Viscograph Data Correlation Version: 4.2.3).

Preparation of pasta

Seven sets of pasta were prepared from the wheat flour, and fortified flours (3, 6, 9, 12, 15 mM and NaFeEDTA) among them wheat flour served as control. The pasta was prepared according to the method explained by Prabhasankar et al. (2009). The fortified wheat flours were hydrated to 30–35% moisture content and mixed in Hobart mixer (Hobart, Ohio, USA) at 59 rpm. The resulting dough was transferred to laboratory scale pasta extruder (Imperia & Monferrina Moncalieri (TO), Italy) fitted with twisted pasta dies having a perforation of 0.7 mm diameter and the resultant pasta was dried in the tray drier at 75 °C for 3 h.

Analysis of pasta (colour, cooking solid loss, texture and sensory analysis)

The pasta was cooked in boiling water at 1:10 (W:V) ratio, optimum cooking time, weight gain by cooked pasta and solid loss during cooking was determined according to the method explained by Gallegos-Infante et al. (2010). The 25 g raw pasta was added to 250 ml boiling water and until its white core was disappeared after being pressed between two glass plates. The cooked gruel was measured for the solids leached from the pasta by evaporating the cooking water in glass Petri dishes at 100 °C. The surface color was measured using hunter lab color flux (Hunter Associates Laboratory Inc., Reston, VA) and reported in terms of lightness (L) and colour (+a: red; –a: green; +b: yellow; –b: blue). Cooked pasta firmness was measured through texture analyzer according to the method used by Prabhasankar et al. (2009). The sensory evaluation of cooked pasta was performed with 20 semi-trained judges; the pasta was scored for appearance (10), strand quality (10), mouthfeel (10) and overall acceptance (30), the

institutional ethical committee approved for performing the sensory analysis of the iron-fortified pasta.

Dialyzable iron

The iron bioavailability in vitro was performed according to the method described by Luten et al. (1996) with slight modifications. The experiment contains two phases viz., gastric and intestinal phase. (1) Gastric phase: 10 g of the fortified flours were mixed in 80 g water in 250 ml capacity conical flask and pH was adjusted to 2 using 6 M HCl. 3 g pepsin solution (16 g pepsin in 100 ml of 0.1 M HCl) was added to flour suspension and made up to 100 g using water and incubated at 37 °C in shaking water bath for two hours. After incubation, the titratable acidity was determined in the pepsin digest using 0.5 M NaOH; titratable acidity is the volume of 0.5 M NaOH consumed to attain a pH of 7.5. (2) Intestinal phase: 20 g of pepsin digest aliquots and dialysis bag containing the equivalent amount of NaHCO₃ (calculated from titratable acidity) in 25 ml water was incubated in the water bath at 37 °C for 0.5 h. Five grams of the pancreatin-bile mixture (4 g pancreatin and 25 g bile extract in 1 lit of 0.1 M NaHCO₃) was added and incubated for 2 h. The dialysis bag was removed washed with de-ionized water, and total iron in the contents of the dialysis bag and the pepsin digest aliquots were analyzed and calculated according to the method explained by Wolfgor et al. (2002). The contents of the dialysis bag or the pepsin digest aliquots were collected in the boiling tube containing HNO₃–HClO₄ (60:40). The total iron analysed through Microwave plasma—Atomic Emission Spectrophotometer (4210 Mp-AES, Agilent Technologies, INC. United States).

The iron dialysability was calculated using the formula:

$$\text{Fe Dialysability (\%)} = [D/(W \times A)] \times 100$$

where *D* is the total amount of dialyzed Fe (μg); *W* is the weight of the food sample in the pepsin digest (g), and *A* is the concentration of Fe in the food (μg/g).

Animals and experimental methods

Adult male Wister rats (weighing 100 ± 10 g) were obtained from the CFTRI animal house facility (Regulated with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), member of the environment, Forests and climate change, Govt of India). The animal ethical committee (IEAC) approved all the experimental procedures. Animals housed in polycarbonate cages with solid floors and rice husk bedding under the controlled temperature (22 ± 2 °C), 12:12 h light: dark cycles and relative humidity was 50 ± 10%. The experiment was carried out according to the method explained by

Table 1 Composition of fortified flours and pasta quality

Sample	Wheat flour (g)	L*	a*	b*	Texture (F)	Cooking water absorption (%)	Cooking loss (%)	Cooking time (min)	Total iron (mg/g)	Dialyzable Fe (%)	Sensory acceptance Overall quality score (30)
Control	–	100	51.4 ± 0.3 ^a	0.3 ± 0.04 ^a	13.4 ± 0.07 ^a	233 ± 1.5 ^a	4.4 ± 0.2 ^a	5.0 ± 0.03 ^a	0.03 ± 0.001 ^a	7.4 ± 0.002 ^a	25 ± 1 ^a
NaFeEDTA	10 mg	100	51.3 ± 0.4 ^b	0.3 ± 0.02 ^b	13.5 ± 0.09 ^a	238 ± 0.9 ^b	4.5 ± 0.3 ^a	5.5 ± 0.04 ^b	0.09 ± 0.002 ^b	12.4 ± 0.001 ^b	27 ± 2 ^a
15 mM	10 g	90	47.3 ± 0.4 ^b	1.4 ± 0.02 ^b	13.5 ± 0.09 ^a	207 ± 0.9 ^c	3.9 ± 0.3 ^a	4.4 ± 0.04 ^c	0.1 ± 0.003 ^b	13.4 ± 0.006 ^b	27 ± 2 ^a
12 mM	14 g	86	43.8 ± 0.3 ^c	1.9 ± 0.05 ^b	12.1 ± 0.06 ^b	204 ± 0.8 ^c	4.4 ± 0.2 ^a	4.3 ± 0.05 ^c	0.1 ± 0.001 ^b	13.9 ± 0.002 ^b	25 ± 2 ^a
9 mM	16 g	84	39.7 ± 0.2 ^d	2.6 ± 0.03 ^c	12.4 ± 0.04 ^b	212 ± 1.2 ^c	4.5 ± 0.1 ^a	4.0 ± 0.02 ^c	0.1 ± 0.006 ^b	13.0 ± 0.004 ^b	23 ± 2 ^a
6 mM	20 g	80	38.8 ± 0.4 ^d	2.9 ± 0.02 ^c	11.5 ± 0.05 ^c	209 ± 0.9 ^c	4.4 ± 0.2 ^a	3.8 ± 0.01 ^d	0.1 ± 0.004 ^b	12.7 ± 0.006 ^b	21 ± 1 ^b
3 mM	40 g	60	36.4 ± 0.2 ^d	3.0 ± 0.01 ^c	11.2 ± 0.02 ^c	224 ± 0.8 ^d	4.3 ± 0.4 ^a	3.5 ± 0.03 ^d	0.1 ± 0.006 ^b	13.1 ± 0.003 ^b	20 ± 1 ^b

Data are presented as mean ± SEM (n = 3)

Means with same superscript letter in a column do not vary significantly ($p < 0.05$) from each other

Whittaker and Vanderveen (1990). The total numbers of 49 animals were grouped into the control group (7 rats) and iron deficient group (42 rats), the control group rats received the AIN-93 N control diet ad libitum. The iron deficient group rats were subjected to depletion and repletion phases. During the depletion phase, iron deficiency was induced by phlebotomy followed by feeding low iron (< 10 ppm iron) AIN semi-purified diet devoid of ferric citrate ad libitum. Deionized water was available ad libitum throughout the experiment. On the 14th—day body weight and hematology (determined using Sysmex XP-300™ Automated Analyzer, Transasia, India) were determined in the experimental animals. The rats were randomized into five groups of seven animals with approximately equal mean body weight and hemoglobin concentration. For the repletion phase, the 15 mM group flour was made to pasta as previously explained (section preparation of pasta). The pasta was cooked, freeze-dried and made to a fine powder. Six diets were prepared by substitution of the pasta powder at 0, 2, 4, 6, 8% and NaFeEDTA (4.5 mg/100 g) in iron-deficient AIN semi-purified diet. Dietary intake and excretion were monitored, gross feed efficiency (GFE), percentage iron absorption (PIA) was determined. The GFE is the ratio of the body weight gain to the weight of the feed intake by the rats during the repletion phase. The PIA was determined as the percentage of iron absorbed from the diet during the repletion phase.

On 28th day rats were euthanized by carbon dioxide asphyxiation and blood was analyzed immediately, liver, kidney, spleen, and heart were collected and weighed. The blood parameters (Haemoglobin (g/dL), Haematocrit (%), Mean corpuscular volume (MCV, fL), Mean corpuscular hemoglobin (MCH, pg) and Mean corpuscular hemoglobin concentration (MCHC, g/dL) were analyzed through hematology analyzer (Sysmex XP-300™ Automated Analyzer, Transasia, India). Serum iron was analyzed using the kit (M/S Agappe Diagnostics, Kerala). Relative iron bioavailability was calculated using hemoglobin regeneration efficiency (HRE). Initial and final hemoglobin iron (mg Hb Fe) of each animal was determined as:

$$g \text{ body wt} \times \frac{6.7 \text{ ml blood}}{100 \text{ g bodywt}} \times \frac{g \text{ hemoglobin}}{100 \text{ ml of blood}} \times \frac{3.35 \text{ mg iron}}{g \text{ hemoglobin}}$$

The efficiency of the treatment was calculated for each animal as follows

$$\frac{(mg \text{ Hb Fe})_{\text{Final}} - (mg \text{ Hb Fe})_{\text{Initial}}}{mg \text{ iron consumed}} \times 100$$

Statistical analysis

All data presented are mean with standard deviation. The one-way analysis of variance followed by Tukey's multiple comparisons test conducted using GraphPad Prism Ver.7.04 (GraphPad Software, Inc. USA) statistical software. *P* values of < 0.01 and < 0.05 was taken significant for in vitro and in vivo respectively.

Results and discussion

Germination rate and total iron content of the abiotic stress treated germinated wheat

The volume, moisture, ash, protein, falling number, and sedimentation value of the wheat grains (*Triticum Aestivum*) were 80 kg/Hl, 8.4%, 1.3%, 12.5%, 720 s and 21 ml respectively. There is a strong inhibition of root elongation than the shoot elongation of the wheat grains germinated at six different experimental concentrations of the FeSO_4 solutions (Fig. 1a, b). The percentage inhibition of root elongation was 80% at 3 mM FeSO_4 concentration further increase in the iron treatment strongly inhibited the root elongation. The inhibition of shoot elongation has a significant linear relationship with the given treatment

(Fig. 1c). The relationship can be expressed through the following regression equation

$$SI_{Fe} = 5.112X + 12.88 (R^2 = 0.911, P < 0.01)$$

where *X* is the experimental concentration (mM) of the FeSO_4 solution and SI_{Fe} is the percentage inhibition of the shoot elongation. From the above regression equation, the half-inhibition dose (ID_{50}) of FeSO_4 treatment on the inhibition of shoot elongation was equal to 7.3 mM. The total iron content in the germinated seeds increased as the abiotic stress treatment increased from 0 to 15 mM. The total iron content ranged from 0.035 to 0.7 mg Fe/g in the treatment groups 0–15 mM respectively (Fig. 1d).

Quality analysis of the fortified flours

The moisture, ash, total protein, falling number, sedimentation value, wet and dry gluten percentage of the wheat was 12.2%, 0.45%, 11.3%, 640 s, 23 ml, 30.57%, and 9.81% respectively. The iron fortified flours were prepared by substituting 3 mM, 6 mM, 9 mM, 12 mM and 15 mM at 40, 20, 16, 14, and 10% respectively in wheat flour to make the final iron concentration of 0.1 mg/g. The effect of fortifying wheat flour with NaFeEDTA or abiotic stress treated flours on falling number, farinograph, extensograph and amylograph were reported (Fig. 2). The falling number showed no changes due to NaFeEDTA fortification in

Fig. 1 Effect of FeSO_4 abiotic stress on germinating wheat **a** Germinated seeds with reduced root and shoots (1–0 mM; 2–3 mM; 3–6 mM; 4–9 mM; 5–12 mM; 6–15 mM). **b** Length of the root and shoot of the germinated wheat. **c** Effect of abiotic stress on inhibition

of the shoot elongation. Data are presented as mean $\pm$ SEM (*n* = 3). **d** Total iron content in the FeSO_4 abiotic stress treated wheat. Different letters superscripts in the same column indicate significant difference (*p* < 0.05)

Fig. 2 Effect of fortification on the farinograph, extensograph and amylograph. **a** Farinograph of the fortified flours; A—Wheat flour (control); B—NaFeEDTA; C—3 mM; D—6 mM; E—9 mM; F—12 mM; G—15 mM. **b** Amylograph of the fortified flours A—Wheat flour (control); B—NaFeEDTA; C—3 mM; D—6 mM; E—9 mM;

F—12 mM; G—15 mM. Data are presented as mean ± SEM ($n = 3$). **c** Farinograph and extensograph parameters of the fortified flours. Different letters superscripts in the same column indicate significant difference ($p < 0.05$)

wheat flour. The 3–15 mM composite flours (AWF) decreased the falling number, which indicates a higher amylase activity. The decrease in the falling number was higher in the 3 mM than in the 15 mM composite flour. The farinograph water absorption, dough development time, dough stability of wheat flour was 59%, 5 min, and 7.5 min respectively. The NaFeEDTA fortification of wheat flour did not have any effect on farinograph characteristics. The 3–15 mM composite flours have higher water absorption than the control wheat flour. Among the composite flours, 3 mM has higher water absorption than the 15 mM. The similar increase in the water absorption was observed in the composite flours prepared by adding germinated chickpeas and kidney peas in wheat flour (Eissa et al. 2007). The bran content in the composite flours has a hygroscopic effect which increased the water absorption. The water absorption capacity of the tested flour depends on the high barn content, damaged starch, and enzyme activity (Hallén et al. 2004; Eissa et al. 2007). The dough development time and stability of the composite flours decreased. The 3 mM flour has less dough stability than the 15 mM flour. The extensograph properties of the fortified flours were illustrated in Fig. 2c. The extensograph

characteristics of the composite flours showed decreased resistance to extension and extensibility than the control or NaFeEDTA fortified wheat flour. The proportion number ($D = R/E$) was much lower for the 3 mM composite flour which indicates the soft and sticky dough than the 15 mM composite flour. The lower R/E indicates the soft/weak dough; the increase in the percentage of the abiotic stress treated flour in the composite flour decreased the R/E ratio which indicates a decrease in dough stiffness. Similar effects on extensibility were observed by Eissa et al. (2007) when germinated legumes were added to wheat flour. Similarly, gluten dilution effect and enzymatic activity affected the germinated cowpea based wheat composite flours dough strength (Hallén et al. 2004).

The germination of the wheat increases the proteolytic and amylolytic enzymes which result in the partial destruction of the protein and carbohydrate networks thus reduces the dough forming ability (Singh et al. 1987). The pasting characteristics of the composite flours were reported in Fig. 2c. The NaFeEDTA fortification of the wheat flour showed no significant effect on the pasting characteristics. The peak viscosity, and set back properties of the composite flours decreased, the decrease was

higher in the 3 mM than in the 15 mM composite flours. The decrease in the peak and setback viscosities indicates the decreased gelatinization and retrogradation respectively due to the degradation of the starch. Similarly, Sekhon et al. (1995) observed decreased peak and setback viscosities due to increased amylase activity in sprouted wheat due to the degradation of the starch. The enzyme activity and gluten dilution due to low gluten in the abiotic stress treated flours affected the dough rheology of the composite flours.

Pasta quality

The cooking quality parameters of the fortified pasta were reported in Table 1. The NaFeEDTA fortification has no effect on the cooking quality parameters. The cooking time for the pasta from composite flours was 3.4 min, which is less than the cooking time of wheat pasta (5.0 min). The cooking time is lower in the 3 mM than in the 15 mM composite flour as the substitution was higher in the 3 mM composite flour. Similar reports were reported by Torres et al. (2007) when germinated pigeon pea flour was substituted at a higher level in wheat pasta. The cooking water absorption was lower in the pasta prepared from the composite flours. These results were in contrast to the results reported by Torres et al. (2007) where they observed higher water absorption on germinated pigeon peas substituted wheat semolina. The higher water absorption might be due to the more protein and bran content in the pigeon peas. The cooking solid loss (%) of the cooked NaFeEDTA fortified wheat pasta or composite flours based pasta showed no significant difference with the cooking solid loss of control pasta (Table 1). The cooking loss was less than 9%, cooking loss above 9% was undesirable in pasta making (Torres et al. 2007). The NaFeEDTA fortification has not affected the color of the pasta. The brightness (L) and yellow (+b) color decreased in the pasta made from composite flours, the reduction in the brightness and yellow color was higher in the 3 mM than in the 15 mM composite pasta. The red (+a) color value increased in the composite pasta; the increase was higher in the 3 mM than in the 15 mM composite pasta. The NaFeEDTA fortification has no effect on the pasta firmness. The pasta firmness had slightly increased in the composite pasta. The sensory acceptability of the cooked pasta is presented in the Table 1, the wheat and NaFeEDTA fortified, 15 mM and 12 mM composite pasta were equally accepted. The acceptability of the 9 mM, 6 mM and 3 mM composite pasta sensory acceptability slightly reduced due to the decrease in the brightness of the pasta.

The analysis of total and percentage dialyzable iron content in the fortified pasta

The total iron content in the germinated seeds increased as the abiotic stress treatment increased from 0 to 15 mM. The total iron content ranged from 0.035 to 0.7 mg Fe/g in the treatment 0 mM and 15 mM respectively (Fig. 1d). The total iron in the wheat flour was 0.04 mg/g, the total iron in the wheat flour was increased by fortification with either NaFeEDTA or abiotic stress treated flours up to 0.1 mg Fe/g. All the fortified flours were extruded to pasta and the percentage dialyzable iron of the fortified pasta was analyzed through in vitro simulated gastric digestion. The percentage dialyzable iron measured through in vitro simulated gastric digestion is a suitable indicator for the relative iron bio-availability (Miller et al. 1981). The percentage dialyzable iron of control pasta and NaFeEDTA fortified pasta was 7.4 and 12.4 respectively (Table 1). The percentage dialyzable iron of the composite flours Viz., 3, 6, 9, 12 and 15 mM in wheat flour was 13, 12.7, 13, 14, and 13% respectively. The percentage dialyzable iron in the wheat flour fortified with the different abiotic stress treated flours (3 mM to 15 mM) did not differ among each other. Similar studies were performed to increase the total iron content of the wheat flour fortified biscuits from 0.48 to 0.88 mg Fe/g using FeSO_4 along with enhancers (citric acid and tartaric acid), they found that FeSO_4 along with the 100 mg of tartaric acid showed maximum improvement in the bioavailable iron (Govindaraj et al. 2007).

In vivo iron bioavailability

Gross food efficiency (GFE) and percentage iron absorption (PIA)

According to the results from in vitro dialyzable iron, the tested concentrations (3, 6, 9, 12, and 15 mM) have no significant difference among each other. Thus the 15 mM composite flour was used for the preparation of the extruded pasta. The dried pasta was cooked in distilled water, freeze-dried and ground to powder. The pasta powder was replaced with the modified iron deficient AIN-semi purified diet at 2–8%. The total iron in the fortified diet ranged from 1.4 to 5.7 mg Fe/100 g. The efficiency of diet in the experimental rats was evaluated through gross food efficiency (GFE). GFE is the ratio of the body weight gain to the amount of feed consumed. The GFE of the D group (anemic rats) was lower than the C group (healthy animals). The GFE among the experimental rats was lower in the D group fed with iron-deficient diet, and higher in the D groups fed pasta. The lower diet consumption observed in the anemic rats fed iron-deficient diet was due to the anorexia, which is a common symptom of the iron

deficiency. Kapoor and Mehta (1993) also reported the decrease in the feed intake, body weight and hemoglobin due to iron deficient diet and phlebotomy. There was an increase in the GFE of the D_{2-8%} groups (anemic rats) as the percentage of the pasta increased in the iron deficient AIN semi-purified diet. The GFE results conclude that the AIN semi-purified diet fortified with pasta powder have more feed efficiency than the iron-deficient diet fed to D group (Table 2). The GFE results were in correlation with the results of percentage iron absorption (Table 2). The percentage iron absorption (PIA) of the D_{2-8%} groups was higher than the C group. The iron deficiency condition in the D_{2-8%} groups promotes the higher iron absorption from the pasta flour fortified iron deficient diet. The PIA increased as the percentage of the pasta increased from 2% (0.014 ppm total iron) to 4% (0.026 ppm total iron) (Table 2). After that, the PIA decreased as the percentage of pasta increased from 4% (0.026 ppm) to 8% (0.056 ppm). Among the anemic rats fed pasta, the PIA was higher in D_{4%} and lower in D_{8%} (Table 2). The decrease in the iron absorption ratio on replacement of the pasta at the percentage higher than 4 indicates that the iron absorption was regulated at the gastrointestinal tract depending on the iron status of the rats. The metabolism of iron was regulated through absorption from the digestive tract depending on the iron status of the organism

(Buchowski et al. 1989). Murray-kolb et al. (2002, 2003) also observed that the iron absorption from the soybean was higher in the iron deficient women. The regulation of the iron uptake prevents the animal from the over-accumulation of the iron in the tissues.

Hematology and haemoglobin regeneration efficiency (HRE) in anemic rats

The blood parameters of the anemic rats were analyzed to evaluate the iron status of the experimental rats. The improvement in the blood parameters in the anemic rats indicates the improvement in the iron status. The hematology reports (Haemoglobin, hematocrit, MCV, and MCH) were significantly ($p < 0.05$) lower in the D group compared to the C group. The hemoglobin was 6.9 g/dl and 13.7 g/dl in D and C groups respectively. The hematocrit was 30% and 50% in the D and C groups respectively (Table 3). The lower hematology reports indicate that the red blood cell number and size were smaller and contained lower hemoglobin per cell in anemic rats (Table 3). The lower blood hemoglobin reduces the oxygen transport to the cells and tissue which hinders the body growth and metabolism. The blood parameters improved in the anemic groups fed pasta (D_{2%} to D_{8%}). D_{2%} and D_{4%} groups have low hemoglobin than the C group but higher than the D

Table 2 Gross food efficiency (GFE), percentage iron absorption (PIA), and haemoglobin regeneration efficiency (HRE) of the experimental rats

	C	D	D _{2%}	D _{4%}	D _{6%}	D _{8%}	D _{NaFeEDTA}
Body weight (g)							
Initial	145.2 ± 6.3	114.27 ± 5.8	119.38 ± 6.59	124.4 ± 4.3	110.9 ± 4.9	120.3 ± 4.2	115.6 ± 6.3
Final	195.4 ± 8.9 ^a	125.5 ± 5.3 ^b	159.02 ± 5.43 ^c	170.2 ± 5.12 ^d	165.1 ± 6.5 ^c	177.2 ± 7.9 ^c	154.3 ± 4.8
Increase in weight	50.1 ± 12.5 ^a	11.3 ± 1.6 ^b	39.63 ± 4.2 ^c	45.8 ± 5.96 ^a	54.17 ± 9.79 ^a	56.9 ± 8.36 ^a	38.7 ± 4.2 ^c
Total food intake (g)	319.4 ± 8.32	209 ± 6.55	247.3 ± 6.15	237.7 ± 4.9	250.2 ± 6.59	249.5 ± 5.41	239.4 ± 6.21
GFE	0.15 ± 0.04 ^a	0.05 ± 0.09 ^b	0.16 ± 0.026 ^c	0.19 ± 0.016 ^a	0.21 ± 0.029 ^a	0.23 ± 0.024 ^a	0.16 ± 0.018 ^c
Iron intake (mg)	12.85 ± 0.33	1.96 ± 0.06	3.6 ± 0.24	6.33 ± 0.19	10.8 ± 0.24	14.2 ± 0.21	10.7 ± 0.16
Fecal excretion (mg)	3.5 ± 0.27	1.8 ± 0.07	0.8 ± 0.07	0.9 ± 0.13	2.9 ± 0.14	4.5 ± 0.17	3.2 ± 0.14
Absorption (mg)	9.32 ± 0.10	0.12 ± 0.04	2.8 ± 0.23	5.4 ± 0.09	7.8 ± 0.17	9.7 ± 0.08	7.5 ± 0.11
PIA	72.6 ± 0.64 ^a	6.42 ± 1.9 ^b	78.38 ± 0.81 ^c	85.3 ± 0.57	72.37 ± 0.82 ^a	68.19 ± 1.5 ^a	70.1 ± 0.6 ^a
Haemoglobin							
Initial	13.3 ± 0.4	7.4 ± 0.4	7.3 ± 0.4	7.3 ± 0.4	7.3 ± 0.4	7.5 ± 0.1	7.3 ± 0.3
Final	13.7 ± 0.2 ^a	6.9 ± 0.17 ^b	9.7 ± 0.2 ^c	12.0 ± 0.2 ^c	13.8 ± 0.2 ^a	13.9 ± 0.2 ^a	13.7 ± 0.4 ^a
HRE (%)	13.1 ± 2.4 ^a	2.7 ± 7.2 ^b	39.64 ± 2 ^c	44.4 ± 1.9 ^a	35.07 ± 2.4 ^a	25.04 ± 0.7 ^a	39.8 ± 1.8 ^c

Data are presented as mean ± SEM (n = 7)

Means with same superscript letter in a row do not vary significantly ($p < 0.05$) from each other

Groups: Control—C and iron deficient—D supplemented with pasta at 2, 4, 6, 8% and NaFeEDTA are D_{2%}, D_{4%}, D_{6%}, D_{8%}, D_{NaFeEDTA} respectively

Table 3 Haematology of the experimental rats

Group of animals	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
C	13.7 ± 0.2 ^a	50.5 ± 2.1 ^a	62.8 ± 3.7 ^a	19.0 ± 0.3 ^a	30.2 ± 1.4 ^a
D	6.9 ± 0.17 ^b	30.3 ± 5.0 ^b	45.9 ± 3.9 ^b	13.9 ± 1.9 ^b	28.2 ± 0.5 ^a
D _{2%}	9.7 ± 0.2 ^c	38.0 ± 2.7 ^c	46.3 ± 1.9 ^b	14.2 ± 0.5 ^b	30.7 ± 0.3 ^a
D _{4%}	12.0 ± 0.2 ^a	43.9 ± 2.9 ^d	50.8 ± 2.9 ^c	16.1 ± 1.4 ^c	31.8 ± 3.2 ^a
D _{6%}	13.8 ± 0.2 ^a	49.8 ± 2.6 ^a	60.5 ± 2.5 ^a	18.5 ± 0.7 ^a	32.6 ± 0.6 ^a
D _{8%}	13.9 ± 0.2 ^a	48.9 ± 3.2 ^a	61.5 ± 2.4 ^a	18.2 ± 1.2 ^a	31.3 ± 0.9 ^a
D _{NaFeEDTA}	13.7 ± 0.15 ^a	49.6 ± 2.0 ^a	61.9 ± 2.2 ^a	18.6 ± 1.8 ^a	30.6 ± 1.2 ^a

Data are presented as mean ± SEM (n = 7)

Means with same superscript letter in a column do not vary significantly ($p < 0.05$) from each other

Groups: Control—C and iron deficient—D supplemented with pasta at 2, 4, 6, 8% and NaFeEDTA are D_{2%}, D_{4%}, D_{6%}, D_{8%}, D_{NaFeEDTA} respectively

group. The D_{6%} and D_{8%} groups had hemoglobin content significantly similar to the C group. The improvement in the hemoglobin along with the improvement in body weight of the iron deficient rats indicates the effectiveness of the iron-fortified diet (Lobo et al. 2011). The hemoglobin regeneration efficiency (HRE) bioassay considers both the blood hemoglobin and body weight. The HRE bioassay is a suitable indicator for the evaluation of the iron bioavailability from the iron-fortified diet (Buchowski et al. 1989). The iron status of the experimental rats has an influence on the HRE bioassay results, the HRE was lower in the D group, and higher in the D groups fed pasta (Table 2). The HRE has improved as the percentage of the pasta increased from 2 to 4%, further increase (4–8%) decreased the HRE results, and these results can be correlated to the results of the PIA. The D_{4%} showed maximum HRE among the anemic groups fed pasta. Thus the

optimal level of iron 0.026 mg/g from abiotic stress treated flour was found to improve the iron status of the anemic rats. Buchowski et al. (1989) observed a similar decrease in the HRE and percentage iron absorption when iron concentration increased from 0.02 to 0.15 mg/g in the iron-deficient diet. Thus the present results suggest that the iron from the abiotic stress treated pasta improves the blood parameters and body weight and thus the HRE of the anemic rats more efficiently.

Serum iron and organ weights of the experimental rats

The serum iron content was 11% lower in the deficient animal group compared to the control animals group, and there is no significant difference among the groups fed the iron-rich diet (Table 4). The iron deficiency in the anemic

Table 4 Serum iron and organ weight of the experimental animals

	C	D	D _{2%}	D _{4%}	D _{6%}	D _{8%}	D _{NaFeEDTA}
Serum iron (µg/100 ml)	347 ± 25.03 ^a	295 ± 18.9 ^c	342.7 ± 22.9 ^a	340.7 ± 30.7 ^a	338 ± 20.3 ^a	345 ± 20.46 ^a	348 ± 18.25 ^a
Liver parameters							
Weight (g)	8.14 ± 0.31	3.88 ± 0.2	5.95 ± 0.24	6.1 ± 0.26	6.68 ± 0.2	7.28 ± 0.28	6.0 ± 0.3
% Body weight	4.16 ± 0.33 ^a	3.00 ± 0.02 ^b	3.5 ± 0.07 ^c	3.85 ± 0.07 ^d	4.05 ± 0.08 ^a	4.11 ± 0.05 ^a	3.87 ± 0.04 ^d
Spleen parameters							
Weight (g)	1.02 ± 0.06	0.41 ± 0.05	0.64 ± 0.06	0.92 ± 0.06	1.07 ± 0.05	1.08 ± 0.06	0.62 ± 0.02
% Body weight	0.52 ± 0.02 ^a	0.321 ± 0.04 ^b	0.4 ± 0.02 ^c	0.51 ± 0.03 ^a	0.51 ± 0.03 ^a	0.53 ± 0.01 ^a	0.40 ± 0.04 ^c
Kidney parameters							
Weight (g)	1.10 ± 0.08	0.75 ± 0.04	0.95 ± 0.03	0.99 ± 0.06	1.22 ± 0.053	1.16 ± 0.06	0.92 ± 0.02
% Body weight	0.567 ± 0.03 ^a	0.58 ± 0.02 ^a	0.58 ± 0.02 ^a	0.55 ± 0.03 ^a	0.58 ± 0.01 ^a	0.57 ± 0.015 ^a	0.59 ± 0.06 ^a
Heart							
Weight (g)	0.66 ± 0.04	0.64 ± 0.05	0.68 ± 0.04	0.7 ± 0.06	0.72 ± 0.07	0.67 ± 0.07	0.67 ± 0.03
% Body weight	0.34 ± 0.02 ^a	0.5 ± 0.03 ^b	0.43 ± 0.03 ^c	0.39 ± 0.04 ^c	0.34 ± 0.03 ^a	0.33 ± 0.02 ^a	0.43 ± 0.02 ^c

Data are presented as mean ± SEM (n = 7)

Means with same superscript letter in a row do not vary significantly ($p < 0.05$) from each other

Groups: Control—C and iron deficient—D supplemented with pasta at 2, 4, 6, 8% and NaFeEDTA are D_{2%}, D_{4%}, D_{6%}, D_{8%}, D_{NaFeEDTA} respectively

rats had affected the organ weights and non-heme iron content when compared to control animals (Table 4). There was a decrease in the liver and spleen weights, and an abnormal increase in heart weight due to iron deficiency. The kidney weight was not affected due to iron deficiency. The results are comparable with the reports of Kapoor and Mehta (1993), where they reported that the iron deficiency in rats affects the organ weights. The increase in heart weight during iron deficiency was due to cardiomegaly (Zielinska-Dawidziak et al. 2014a). There was an improvement in organ weights of the iron deficient animals fed pasta powder 2–8%. The liver and spleen weight increased in the anemic rats fed iron-rich diet, and the improvement was higher in the groups fed pasta at 6%. The kidney weight was not affected in the experimental rats fed different iron-rich diet. The heart weight decreased on improvement in the iron status of the anemic rats. The supplementation of the pasta at > 4% improve the weight of the vital organs of the iron-deficient rats.

Conclusion

The present study concludes that the FeSO_4 abiotic stress treatment to the germinating wheat increases the iron content which has an inhibition effect on the shoot and root elongation. There is a linear relationship between the FeSO_4 treatment to the seeds and the inhibition of roots and shoots. The root elongation was strongly inhibited at 6 mM treatment. The half-inhibition dose (ID_{50}) of FeSO_4 treatment on the inhibition of shoot elongation was equal to 7.3 mM. The NaFeEDTA fortification to wheat flour showed no effect on farinograph and extensograph and amylograph properties. In 3–15 mM composite flours the farinograph water absorption increased whereas the dough stability, dough extensibility, pasting viscosity and falling number decreased. The decrease in the falling number and pasting viscosity indicate high amylase activity in the composite flours. The pasta prepared from the iron fortified flours showed acceptable cooking and the sensory acceptance, the fortification has no effect on the pasta quality. The dialyzable iron from NaFeEDTA fortified pasta or 3–15 mM composite pasta showed no significant difference between the groups. The in vivo iron bioavailability study conducted using 15 mM abiotic stress treated flour based pasta in iron-deficient diet showed improvement in the hematology, HRE and PIA. The pasta at 4% (Fe—0.026 mg/g) showed PIA and HRE of 85.3% and 44.4% respectively in anemic rats which were higher than the iron deficient rats fed NaFeEDTA. Thus abiotic stress treated flour can be the cost-effective food fortificant than the NaFeEDTA iron salt for the improvement of the iron status in the iron deficient population.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

Ethical approval The institutional ethical committee approved all the experimental procedures involved in the presented experiments.

References

- AACC, 2000 AACC (2000) American Association of Cereal Chemists. Approved methods (10th ed.). St. Paul, Minnesota
- Ahmad N, Kalakoti P, Bano R, Aarif SM (2010) The prevalence of anaemia and associated factors in pregnant women in a rural Indian community. *Aust Med J* 3(5):276–280
- Buchowski MS, Mahoney AW, Kalpalathika MP (1989) Nonheme iron absorption, apparent iron absorption and haemoglobin regeneration efficiency in anaemic and normal rats fed with dietary heme and nonheme iron at various levels. *Nutr Res* 9(7):773–783
- Cakmak I, Yilmaz A, Kalayci M, Ekiz H, Torun B, Ereno B, Braun HJ (1996) Zinc deficiency as a critical problem in wheat production in Central Anatolia. *Plant Soil* 180(2):165–172
- Dharmalingam A, Navaneetham K, Krishnakumar CS (2010) Nutritional status of mothers and low birth weight in India. *Matern Child Health J* 14(2):290–298
- Eissa HA, Hussein AS, Mostafa BE (2007) Rheological properties and quality evaluation on Egyptian balady bread and biscuits supplemented with flours of ungerminated and germinated legume seeds or mushroom. *Pol J Food Nutr Sci* 57(4):487–496
- Gallegos-Infante JA, Rocha-Guzman NE, Gonzalez-Laredo RF, Ochoa-Martínez LA, Corzo N, Bello-Perez LA, Peralta-Alvarez LE (2010) Quality of spaghetti pasta containing Mexican common bean flour (*Phaseolus vulgaris* L.). *Food Chem* 119(4):1544–1549
- Govindaraj T, KrishnaRau L, Prakash J (2007) In vitro bioavailability of iron and sensory qualities of iron-fortified wheat biscuits. *Food Nutr Bull* 28(3):299–306
- Hallén E, İbanoğlu Ş, Ainsworth P (2004) Effect of fermented/germinated cowpea flour addition on the rheological and baking properties of wheat flour. *J Food Eng* 63(2):177–184
- Hurrell R, Ranum P, de Pee S, Biebinger R, Hulthen L, Johnson Q, Lynch S (2010) Revised recommendations for iron fortification of wheat flour and an evaluation of the expected impact of current national wheat flour fortification programs. *Food Nutr Bull* 31(1):S7–S21
- Kalgaonkar S, Lonnerdal B (2009) Receptor-mediated uptake of ferritin-bound iron by human intestinal Caco-2 cells. *J Nutr Biochem* 20(4):304–311
- Kapoor R, Mehta U (1993) Iron status and growth of rats fed different dietary iron sources. *Plant Foods Hum Nutr* 44(1):29–34
- Kloots W, Op den Kamp D, Abrahamse L (2004) In vitro iron availability from iron-fortified whole-grain wheat flour. *J Agric Food Chem* 52(26):8132–8136
- Lobo AR, Gaievski EHS, Colli C (2011) Haemoglobin regeneration efficiency in anaemic rats: effects on bone mineral composition and biomechanical properties. *Biol Trace Elem Res* 143(1):403–411

- Lucca P, Hurrell R, Potrykus I (2001) Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. *Theor Appl Genet* 102(2):392–397
- Lucca P, Hurrell R, Potrykus I (2002) Fighting iron deficiency anemia with iron-rich rice. *J Am Coll Nutr* 21(3):184S–190S
- Luten J, Crews H, Flynn A, Van Dael P, Kastenmayer P, Hurrell R, Farre R (1996) Interlaboratory trial on the determination of the in vitro iron dialysability from food. *J Sci Food Agric* 72(4):415–424
- Martinez-Navarrete N, Camacho MM, Martinez-Lahuerta J, Martinez-Monzo J, Fito P (2002) Iron deficiency and iron fortified foods—a review. *Food Res Int* 35(2):225–231
- Miller DD, Schricker BR, Rasmussen RR, Van Campen D (1981) An in vitro method for estimation of iron availability from meals. *Am J Clin Nutr* 34(10):2248–2256
- Murray-Kolb LE, Takaiwa F, Goto F, Yoshihara T, Theil EC, Beard JL (2002) Transgenic rice is a source of iron for iron-depleted rats. *J Nutr* 132:957–960
- Murray-Kolb LE, Welch R, Theil EC, Beard JL (2003) Women with low iron stores absorb iron from soybeans. *Am J Clin Nutr* 77(1):180–184
- Muthayya S, Thankachan P, Hirve S, Amalrajan V, Thomas T, Lubree H, Kurpad AV (2012) Iron fortification of whole wheat flour reduces iron deficiency and iron deficiency anemia and increases body iron stores in Indian school-aged children—4. *J Nutr* 142(11):1997–2003
- Prabhasankar P, Ganesan P, Bhaskar N, Hirose A, Stephen N, Gowda LR, Hosokawa M, Miyashita K (2009) Edible Japanese seaweed, wakame (*Undaria pinnatifida*) as an ingredient in pasta: chemical, functional and structural evaluation. *Food Chem* 115(2):501–508
- Sachdeva B, Kaushik R, Arora S, Kapila S (2015) Bioavailability of iron in multiple fortified milk. *J Food Sci Technol* 52(9):6017–6023
- Sekhon KS, Singh N, Kaur H, Nagi HPS (1995) Improving the functional and bread making properties of sprouted Indian wheat. *J Food Process Preserv* 19(2):147–160
- Shaw NS, Liu YH (2000) Bioavailability of iron from purple laver (*Porphyra* spp.) estimated in a rat haemoglobin regeneration bioassay. *J Agric Food Chem* 48(5):1734–1737
- Singh N, Sekhon KS, Nagi HPS (1987) Laboratory sprout damage and effect of heat treatment on milling and baking properties of Indian wheats. *J Food Sci* 52(1):176–179
- Singh H, Singh N, Kaur L, Saxena SK (2001) Effect of sprouting conditions on functional and dynamic rheological properties of wheat. *J Food Eng* 47(1):23–29
- Torres A, Frias J, Granito M, Vidal-Valverde C (2007) Germinated *Cajanus cajan* seeds as ingredients in pasta products: chemical, biological and sensory evaluation. *Food Chem* 101(1):202–211
- Wang M, Zhou Q (2005) Single and joint toxicity of chlorimuron-ethyl, cadmium, and copper acting on wheat *Triticum aestivum*. *Ecotoxicol Environ Saf* 60(2):169–175
- Whittaker P, Vanderveen JE (1990) Effect of EDTA on the bioavailability to rats of fortification iron used in Egyptian balady bread. *Br J Nutr* 63(3):587–595
- Wolfgor R, Drago SR, Rodriguez V, Pellegrino NR, Valencia ME (2002) In vitro measurement of available iron in fortified foods. *Food Res Int* 35(1):85–90
- Zielinska-Dawidziak M, Hertig I, Piasecka-Kwiatkowska D, Staniek H, Nowak KW, Twardowski T (2012) Study on iron availability from prepared soybean sprouts using an iron-deficient rat model. *Food Chem* 135(4):2622–2627
- Zielinska-Dawidziak M, Hertig I, Staniek H, Piasecka-Kwiatkowska D, Nowak KW (2014a) Effect of iron status in rats on the absorption of metal ions from plant ferritin. *Plant Foods Hum Nutr* 69(2):101–107
- Zielinska-Dawidziak M, Piasecka-Kwiatkowska D, Warchalewski JR, Makowska A, Gawlak M, Nawrot J (2014b) Sprouted wheat grain with ferritin overexpression as a potential source of iron for cereal product fortification. *Eur Food Res Technol* 238(5):829–835

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