

Relative bioavailability of iron in Bangladeshi traditional meals prepared with iron-fortified lentil dal



Fortified lentil



<http://www.taste.com.au/recipes/14107/lentil-dhal>



http://news.bbc.co.uk/2/hi/south_asia/7445570.stm



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Outline of presentation

1. Background of this study
2. Materials and methods
3. Results
4. Conclusion
5. Future research

Iron (Fe)



Most abundant mineral on Earth and the most abundant trace mineral in the body

Iron deficiency = most common nutrient deficiency in world

Fe and its Deficiency

Major consequences of iron deficiency
(Hope, et.al., 2008)



Anemia



Decreased aerobic performance



Thermoregulation disorders



Fatigue



Maternal and child mortality



Immunes system alterations



Altered cognitive functions

Causes of Fe Deficiency

➤ Nutritionally unbalanced food supply



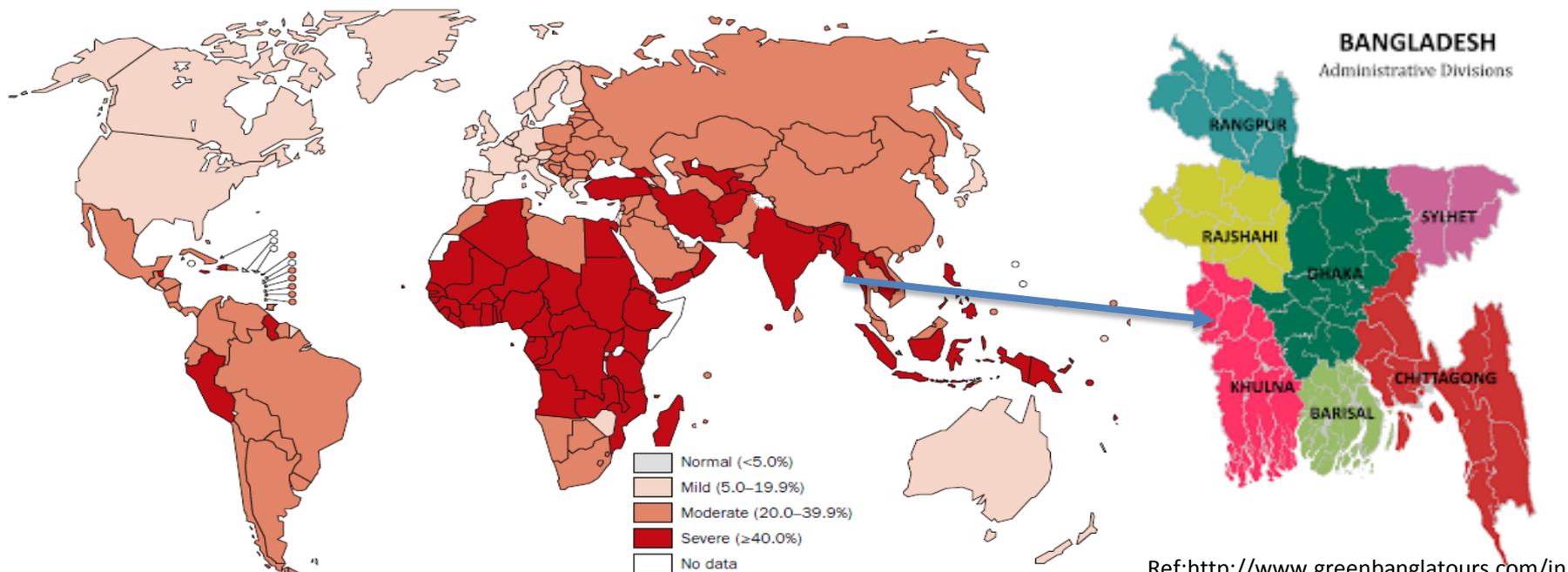
➤ Food habits



➤ Socio-economics (poverty)



Anemia Prevalence Worldwide



Ref.: http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf?ua=1

Ref: <http://www.greenbanglatours.com/index.php?action=page&id=17>

- ❑ >60% preschool aged children and > 40% pregnant and non pregnant women in South east Asia and Africa are suffering from Fe deficiency anemia, [WHO, 2008]
- In Bangladesh, 40% of adolescents are anaemic [Ahmed et al., 2010].
- In 2011, the national prevalence of anaemia in Bangladesh was 51% in children aged 6-59 months and 42% in non-pregnant women [BDHS, 2011].

Lentil (*Lens culinaris* Medik.) – a carrier of iron

- ❑ Lentil is the sixth most important pulse crop
- ❑ Good source of protein, fiber, minerals, vitamins, and antioxidants
- ❑ Excellent source of micronutrients (Zn, **Fe**, and Se) [*Thavarajah et al. 2011*]
- ❑ **Saskatchewan** is the world's largest lentil producer and exporter



Fe improvement in lentil

Biofortification

The process by which the **nutritional quality of food crops** is improved through agronomic practices, conventional plant breeding, or modern biotechnology. (WHO, 2016)

Fortification

The practice of **deliberately increasing the content** of an essential micronutrient, i.e. vitamins and minerals,“ (WHO and FAO, 2005)



Vasconcelos et al, 2016



Parker, 2013

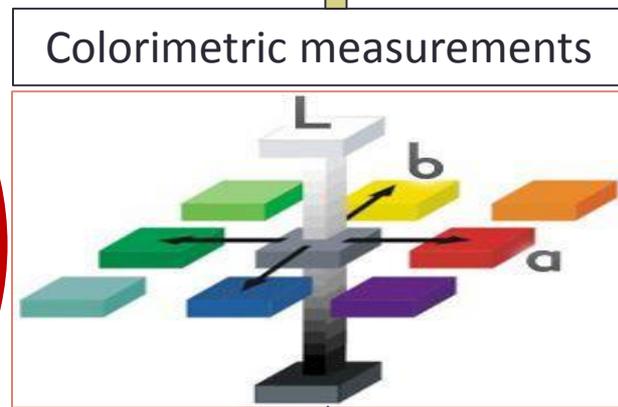
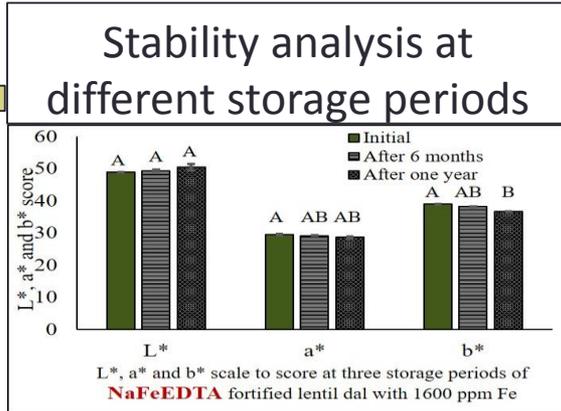
Fortification of lentil



Unfortified lentil



Fortified lentil



Appropriate dose of Fe solution to address RDA for humans



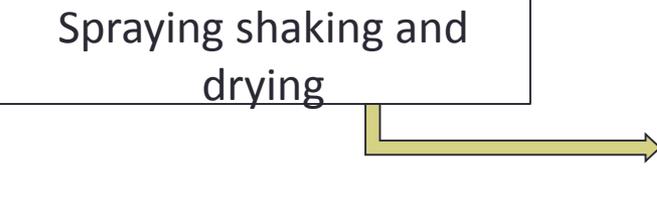
Selection of Fe fortificant

Appropriate method selection



Spraying shaking and drying

Estimation of Fe conc. in fortified lentil



Identification of the optimum Fe fortificant for dehulled lentils

A manuscript was submitted in “Nutrients” Journal and published on August 2017.



nutrients



Article

Iron Fortification of Lentil (*Lens culinaris* Medik.) to Address Iron Deficiency

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and Albert Vandenberg ^{1,*}

Sensory evaluation

9

Objective: Determine sensory acceptability of fortified lentils – appearance, odour, texture, taste and Overall acceptability

University of Saskatchewan



45 Panellists were recruited from staff and students at **U of S** (2 replications)

Bangladesh (BRAC University)

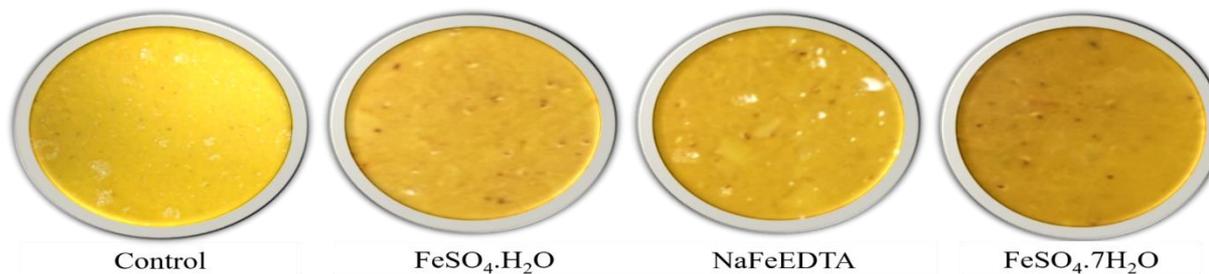


98 consumers were selected

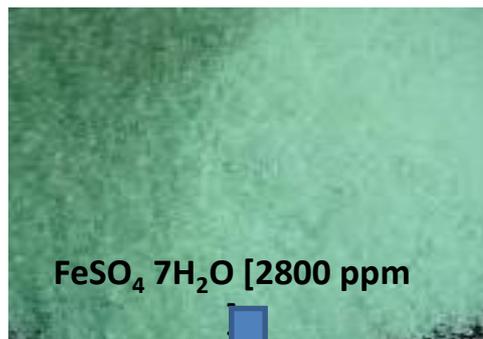
Comparing Uncooked fortified lentil samples



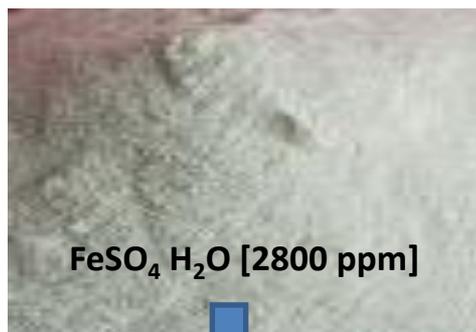
4 cooked samples (fortified with 1600 ppm Fe)



Outcome of the Sensory evaluation



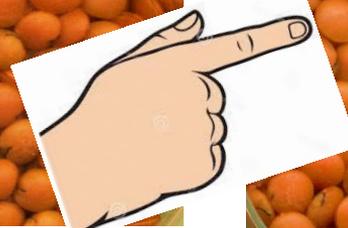
FeSO₄ 7H₂O [2800 ppm]



FeSO₄ H₂O [2800 ppm]



NaFeEDTA [2800 ppm]



Journal of Food Science

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Early View

Sensory & Food Quality

Sensory Acceptability of Iron-Fortified Red Lentil (*Lens culinaris* Medik.) Dal

Rajib Podder, Shaan M. Khan, Bunyamin Tar'an, Robert T. Tyler, Carol J. Henry, Chowdhury Jalal, Phyllis J. Shand, Albert Vandenberg

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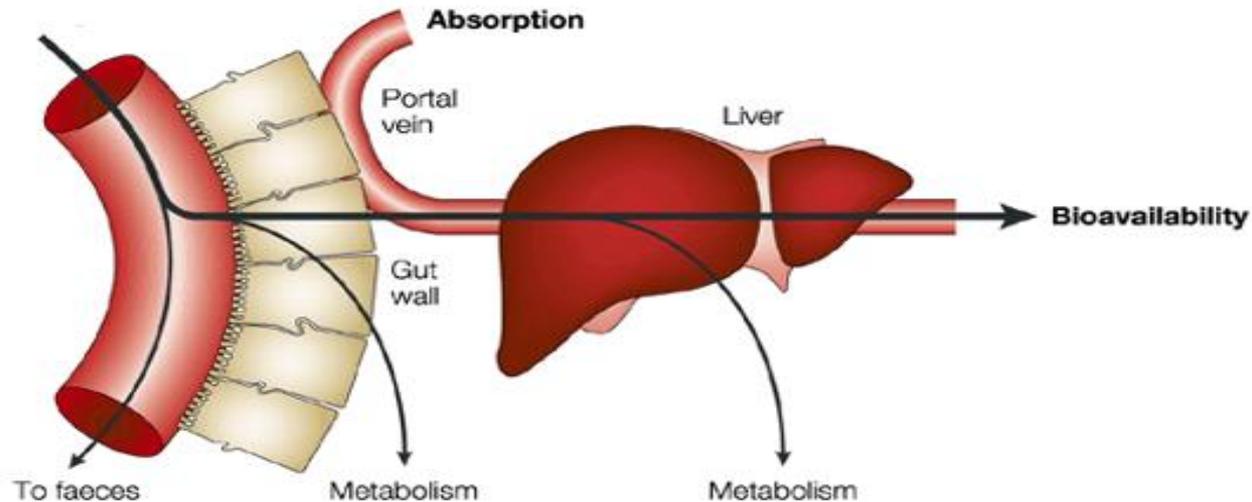


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Bioavailability of Fe from lentil

Bioavailability - is a post-absorption assessment of how much of a nutrient that has been absorbed becomes functional to the system

Source: <https://www.tamu.edu/faculty/.../Lecture%2009%20Bioavailability.ppt>



<https://canna-pet.com/first-pass-effect/>

Objective

To determine the concentration and relative bioavailability of Fe in different traditional Bangladeshi meal plan models featuring fortified and unfortified lentil dal.

Preparation of meal samples with fortified and unfortified lentil dal

Meal ingredients	Meal models														
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
Rice (%)	50	50	75	75	75	75	85	85	85	85	85	75	50	85	50
Vegetable (%)	0	25	10	5	0	0	10	5	0	0	0	25	25	25	0
Fish (%)	0	0	0	10	0	0	0	5	0	5	10	0	25	0	0
Unfortified lentil dal (%)	50	25	15	10	25	25	5	5	15	10	5	0	0	0	0
Fortified lentil dal (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50

Meal ingredients	Meal models														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Rice (%)	50	50	75	75	75	75	85	85	85	85	100	0	0	0	0
Vegetable (%)	25	10	5	0	0	10	5	0	0	0	0	100	0	0	0
Fish (%)	0	0	10	0	10	0	5	0	5	10	0	0	100	0	0
Unfortified lentil dal (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
Fortified lentil dal (%)	25	15	10	25	15	5	10	15	15	5	0	0	0	0	100

Materials and Methods

Meal (Cooked)	Ingredients	Ratio (by weight)
Lentil dal (fortified and unfortified)	lentil, deionized water, canola oil, salt, turmeric powder and onion	15:70:4:3:2:6
Rice	Rice and water (white boiled and unenriched)	
Vegetables	carrot, cauliflower, brinjal, potato, and sweet potato, onion, salt, turmeric, garlic, oil, and water	10:10:8:10:5:2 :1:1:1:12:40
Fish	fish fillets, salt, turmeric, and oil	90:2:3:5

Preparation of Meal Components

- All foods were cooked with 18 MΩ deionized water.
- Rice, fish, and vegetables were cooked in a traditional Bangladeshi fashion.
- Stainless steel cookware was used to prepare all meal components.
- Prepared dishes were cooled at room temperature for 2 h,
 - ❖ Frozen at -80° C for 24 h,
 - ❖ Freeze-dried using a FreeZone 12 L Console Freeze Dry System for 72 h,
 - ❖ Stored at room temperature



Laboratory: Dr. Raymond Glahn, USDA-ARS, Ithaca, New York, Using an *in vitro* digestion/Caco-2 cell culture bioassay (Glahn, 2009).

Iron concentration ($\mu\text{g g}^{-1}$)

Inductively coupled argon-plasma
emission spectrometer



https://en.wikipedia.org/wiki/Inductively_coupled_plasma_mass_spectrometry

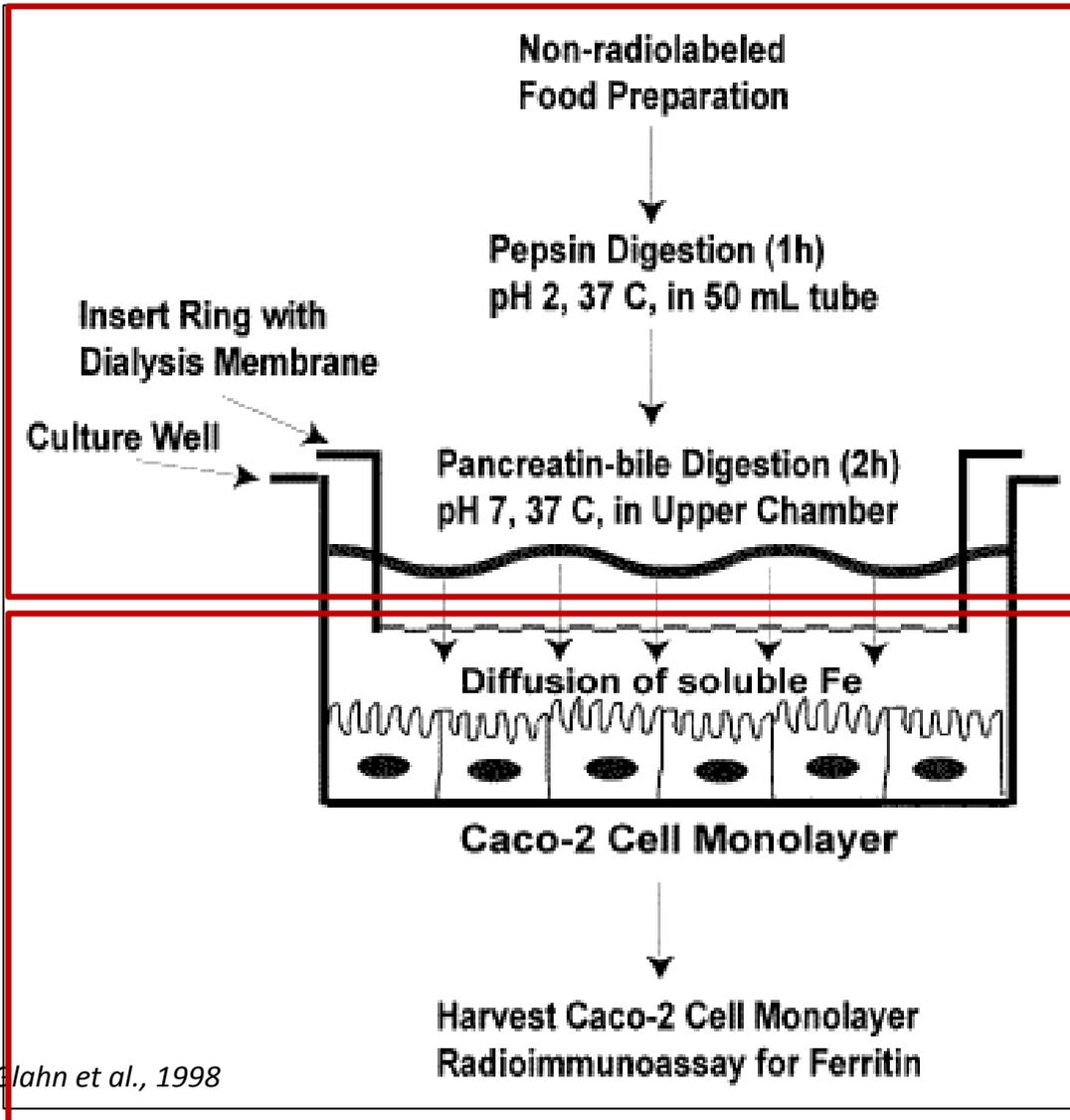
Phytic acid concentration (mg g^{-1})

A colorimetric assay kit (K-PHYT 12/12,
Megazyme International, Wicklow, Ireland)



<https://secure.megazyme.com/myo-Inositol-Assay-Kit>

Relative Fe bioavailability estimation



The Caco-2 Cell Bioassay

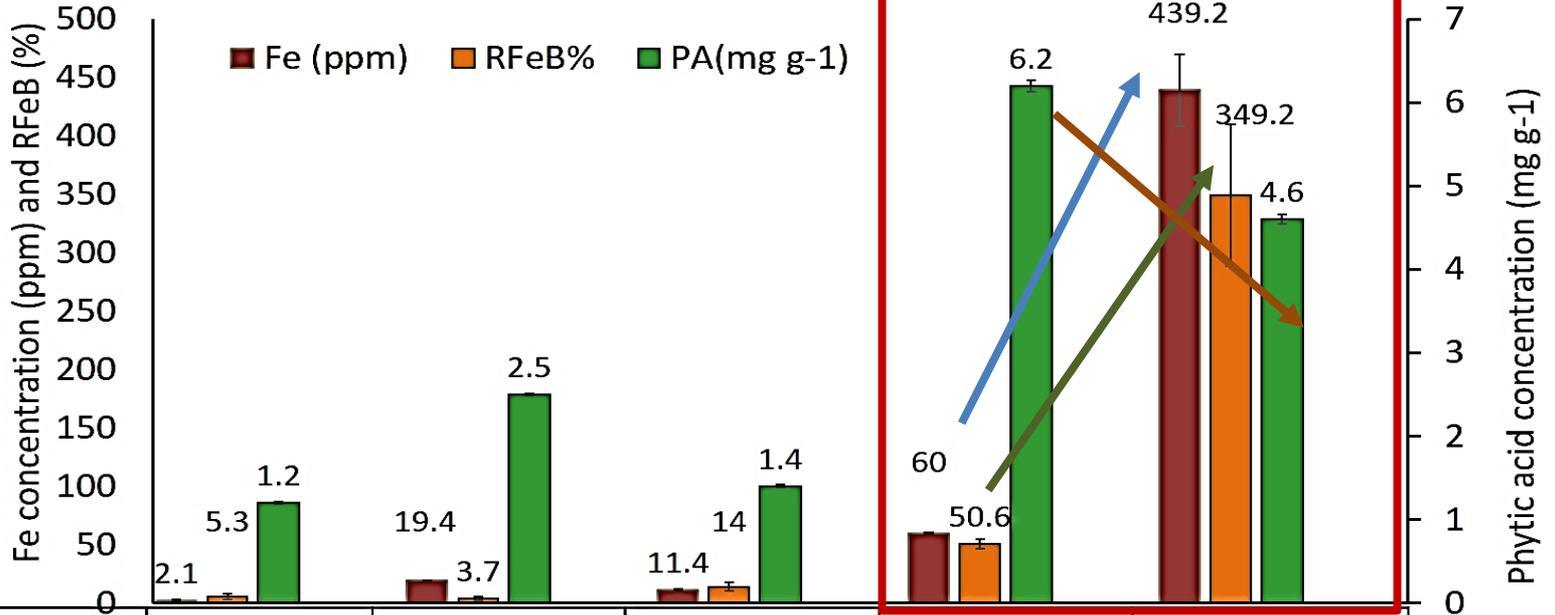
The Caco-2 cell line is a *continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells in intestine*

Relative Fe bioavailability estimation

Relative bioavailability- *is used to rank the absorbability of a nutrient by comparing its absorbability with that of a reference nutrient that is considered as having the most efficient absorbability. [WHO, 2006]*

$$\text{Relative Fe bioavailability (RFeB \%)} = \frac{\text{(ng ferritin of the lentil sample/mg protein of the lentil sample)}}{\text{ng ferritin/mg protein of the control lentil}} \times 100$$

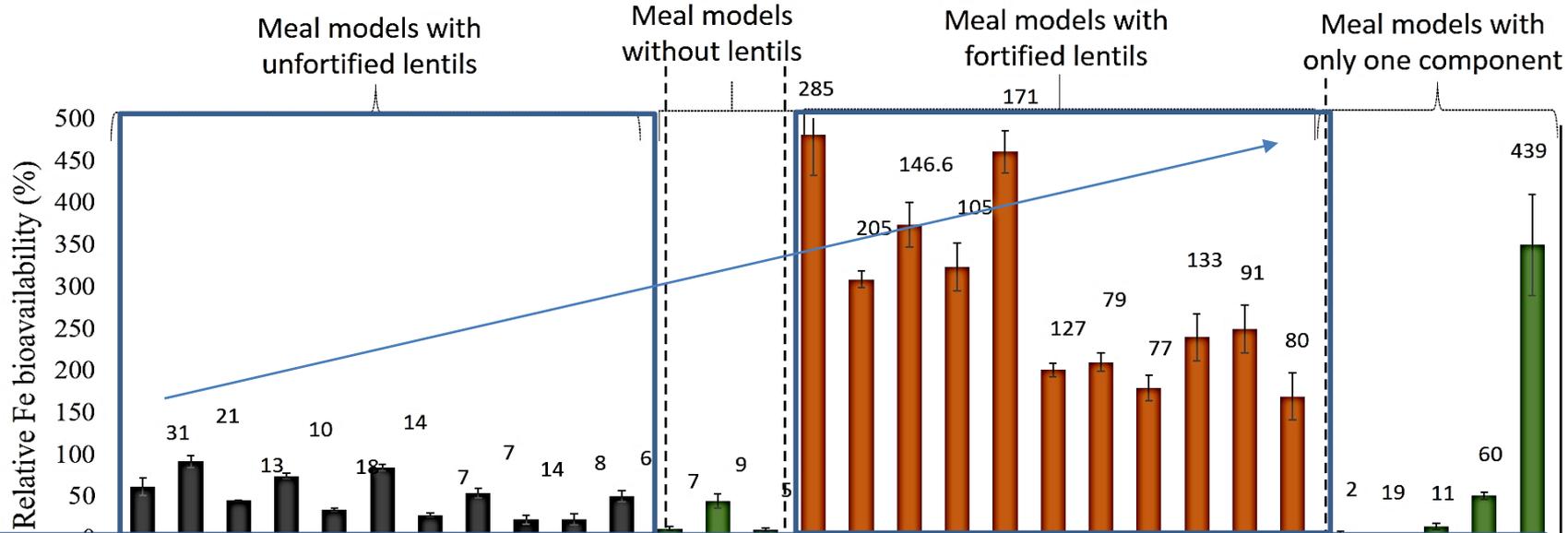
Results



Meal models	Model 26	Model 27	Model 28	Model 29	Model 30
Rice (%)	100	0	0	0	0
Veg (%)	0	100	0	0	0
Fish (%)	0	0	100	0	0
Unfortified lentil dal (%)	0	0	0	100	0
Fortified lentil dal (%)	0	0	0	0	100

Fe concentration ↑ Relative Fe bioavailability ↑
Phytic Acid Conc. ↓ in fortified lentils

Relative Fe bioavailability ↑ in fortified lentils



Meal models	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Rice (%)	50	50	75	75	75	75	85	85	85	85	85	75	50	85	50	50	75	75	75	75	75	75	85	85	85	85	100	0	0	0	0
Vegetable (%)	0	25	10	5	0	0	10	5	0	0	0	25	25	15	0	25	10	5	0	0	10	5	0	0	0	0	100	0	0	0	
Fish (%)	0	0	0	10	0	0	0	5	0	5	10	0	25	0	0	0	0	10	0	10	0	5	0	5	10	0	0	100	0	0	
Unfortified lentil dal (%)	50	25	15	10	25	25	5	5	15	10	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	
Fortified lentil dal (%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	25	15	10	25	15	15	5	15	10	5	0	0	0	0	100	

Fortified lentil Vs Unfortified lentil

Meal model	Fe (ppm)	Ferritin formation (ng ferritin/mg protein)	RFeB (% control lentil)	PA (mg/g)	PA: Fe molar ratio
Meals with unfortified lentil (models one to 11) (n = 11)	13.5±7.5	15.9±7.5	51.2±24.2	2.4±0.7	111.4±32.9
Meals with fortified lentil (model 15 to 25) (n = 11)	136.3±64.3	52.5±25.2	289.9±109.3	2.1±0.3	9.6±3.02
p	< 0.001	< 0.001	< 0.001	0.03	< 0.001

Fe concentration, ferritin formation (ng ferritin/mg protein), relative Fe bioavailability ↑ in meals with fortified lentil

Phytic acid and phytic acid:Fe molar ratio ↑ in meals unfortified lentil

Correlation Coefficients

- Iron (Fe) concentration vs. relative Fe bioavailability (RFeB%), ✓
- Bioavailability vs. phytic acid (PA):Fe molar ratio, and ✓
- Fe concentration vs. PA:Fe molar ratio ✓

Meal model	[Fe] vs. RFeB%	RFeB% vs. PA:Fe molar ratio]	[Fe] vs. PA:Fe molar ratio
All (models 1 to 30) (n = 30)	0.832** (< 0.001)	-0.722** (< 0.001)	-0.627** (< 0.001)
Unfortified lentil (models 1 to 11) (n = 11)	-0.142 (0.685)	0.351 (0.299)	-0.628* (0.0364)
Fortified lentil (model 15 to 25) (n = 11)	0.801** (0.001)	-0.763** (0.004)	-0.628* (0.036)

Conclusion

- Fortification with NaFeEDTA increased the iron concentration in lentil from 60 to 439 $\mu\text{g g}^{-1}$
- The relative Fe bioavailability of cooked fortified lentil was increased by 79% compared to unfortified cooked lentil
- Phytic acid levels were reduced from 6.2 to 4.6 mg g^{-1} when fortified lentil was added
- PA:Fe molar ratio was reduced from 8.8 to 0.9
- Fortified lentil can contribute significant bioavailable Fe to populations at risk of Fe deficiency.

Future research

- Efficacy trial with Fe-fortified lentil

- In-vivo study of fortified lentil

A small-scale study using *in-vivo* could be conducted to validate the results of the in-vitro procedure.

- Multiple fortification of lentil

Studies can be developed to fortify lentil with multiple fortificants to simultaneously mitigate multiple nutrients deficiencies in humans.

Acknowledgements

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Thank you for your attention

