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Original article

Effect of different cooking methods on loss of iron and zinc micronutrients in fortified and non-fortified rice

M. Mohibbe Azam [a,*], Sarla Padmavathi [a], R. Abdul Fiyaz [a], Amtul Waris [a], K.T. Ramya [b], Chirravuri N. Neeraja [a]

[a] ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad 500030, Telangana State, India
[b] ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad 500030, Telangana State, India

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ABSTRACT

Malnutrition is considered as major public health concern and is emerging challenge to food and nutrition security particularly in developing countries. Rice is the staple food and consumed by the half of the world's population which is the source of daily requirement of the nutrients. Attempts are being made to fortify rice with micronutrients, but the loss or retention of these micronutrients in different cooking methods is not well studied and documented especially in fortified rice. In the present study, paddy seeds of six Indian varieties were fortified with iron and zinc by parboiling process. Consequently, fortified polished rice had higher micronutrient contents (Fe, 106.31 ± 12.56; Zn, 97.72 ± 9.75) than non-fortified polished rice (Fe, 7.44 ± 1.05; Zn, 14.74 ± 2.94) expressed in ppm. Polished rice of both fortified and non-fortified were cooked under five different cooking conditions and analyzed for remaining iron and zinc content. Cooking rice in rice cooker without prior washing (NRC) retained highest concentration of Fe and Zinc in both fortified and non-fortified rice varieties. It also showed that fortified rice suffered higher percentage loss of micronutrient, than the non-fortified rice. But the average retained micronutrient amount measured in ppm, was higher in fortified rice (Fe, 43.54 ± 6.88; Zn, 36.7 ± 3.12) than in non-fortified rice (Fe, 4.24 ± 0.87; Zn, 9.3 ± 2.11). Hence, adopting appropriate cooking method, higher amount of micronutrients will be retained in the cooked food which will in turn help in combating the malnutrition and improve health.

1. Introduction

Micronutrients play vital role in various physiological functions. Of these micronutrients, iron and zinc are important for maintaining normal health of an individual and their deficiency constitutes a public health concern (Shenkin, 2006). Consequences of iron malnutrition include poor mental development, lower cognitive ability in preschool children, and increased mortality of mother and child at birth (Stoltzfus et al., 2003; NFHS-4, 2016; WHO, 2006). Predominant health outcomes associated with zinc deficiency include short stature, impaired immune function, and other disorders, like respiratory infections, malaria, diarrhoea, smell and taste failure (Chasapis et al., 2012; Yagi et al., 2013). Global Nutrition Report (2018) discloses that more than 30.9% cases of world's stunted children under the age of five are found in India which is highest in the world.

Insufficient intake of iron and zinc is one of the most significant reasons for the development of their deficiency (de Benoist et al., 2007). As per the report of Food Safety Standards Authority of India (FSSAI), over 70% of India's population still consumes less than 50% of Recommended Dietary Allowance (RDA) for micronutrients (FSSAI, 2017). The prevalence of Fe/Zn deficiency is more common among populations who consume rice (Banerjee et al., 2010; Dexter, 1998) which is a staple food of more than half of the population of the world including India. In 2018, per capita rice consumption in India is very high and stands at 196.6 kg/person/year (Rice Industry Outlook, 2018). While, world's per capita rice consumption is 53.7 kg/person/year (Shahbandeh, 2019). The prevalence of deficiencies of these micronutrients may be due to poor content of iron (~2 ppm) and zinc (~16 ppm) in normal

* Corresponding author.
E-mail address: mohibbe.azam@gmail.com (M. Mohibbe Azam).
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polished grains of widely grown rice varieties (Bouis et al., 2011; Trijatmiko et al., 206). Consumption of fortified rice appears to be the best long-term nutrition intervention strategy for combating iron and zinc deficiencies. Currently, four types of fortification technology for rice are in use namely hot extrusion, cold extrusion, coating and dusting in the countries like China, the USA, the Philippines, and Costa Rica as reported by Alavi et al. (2008). Dusting rice with electrostatically-attracted mineral powders is largely considered ineffective; as rice washing methods commonly practiced in India to remove adulterants also remove micronutrients (Alavi et al., 2008). In coating process of fortification, high concentration of micronutrients is added to a fraction of rice and subsequently, rice kernels are coated with water resistant edible coatings and then mix with normal polished rice. Fortified rice obtained through the process of dusting or coating is not suitable for cooking with excess of water which is discarded after cooking.

Extrusion process (cold, warm or hot) of fortification involves several steps like preparation of rice flour, addition of fortificants and other additives including water to make dough, then extrusion through a rice shape die. The rice pieces thus formed are optionally dusted with cross linking agent and then dried. Fortified kernels are then blended with normal polished rice but unpalatable fortified kernels are easily identified and removed due to their appearance (Alavi et al., 2008). Moreover, these fortified grains may have off taste/metallic taste. It is likely that fortified reconstituted grains are not uniformly mixed with non-fortified rice. Therefore, some portion of the mixed rice can have more fortified grains and other portion can have less. But fortification through the methods of wax coating, cold extrusion with a binding agent and hot extrusion requires much greater capital equipment costs to produce thus, these methods are challenged by resources available (Alavi et al., 2008; Wieringa et al., 2014). Biofortification is an alternative method which can improve the nutritional content of staple food (Bouis et al., 2011). Till date only 3 biofortified zinc rice varieties have been developed and released in Bangladesh and India (Saltzman et al., 2016). One of the limitations of biofortified staple food is- it cannot provide as high a level of micronutrients as supplements or industrially fortified food. Among the physicochemical methods of fortification, high investment cost has been identified as one of the major constraints to scaling up rice fortification (Piccoli et al., 2012).

On the other hand, fortification of rice with iron and zinc through parboiling process is more cost-effective and economically more viable than other methods as fortification can be carried out with the existing parboiling facilities (Prom-u-thai et al., 2011). In this process of fortification each and every grain is fortified and the cost of the fortified rice will be slightly higher than of parboiled rice due to the cost of added fortificants. At present, 60% of rice mills in India are linked with parboiling units out of 1.3 lakhs mills around the country. (Singaravadivel, 2016). Moreover, parboiled rice exhibits several advantages over raw rice such as increased head rice recovery, higher nutritional values, improved shelf life, and higher recovery of oil (Kumar et al., 2018). Although parboiling of rice results in considerable loss of zinc in the polished rice grains (Chukwu and Oseh, 2009) but fortification of rice with iron and zinc through parboiling may result in 5- folds increase in the concentration of iron and zinc in rice kernels (Prom-u-thai et al., 2011). Information on effects of different cooking methods on retention of iron and zinc in such fortified rice grain is very limited (Wieringa et al., 2014). Similarly, very few cooking methods have been tested for retention of iron and zinc in normal polished rice (Khatoon and Prakash, 2006; Mwale et al., 2018).

This paper aims to quantify the amount of iron and zinc retained in fortified rice as well as in non-fortified rice cooked by commonly used cooking methods in different parts of the country. With this objective, six different rice varieties were first fortified with iron and zinc through parboiling process and then evaluated for the effects of five different cooking methods on the content of iron and zinc in cooked fortified rice as well as in cooked non-fortified rice of same six rice varieties. This will help in identifying the most appropriate method of cooking for retaining maximum amount of these micronutrients. The generated information can be used to educate the rice consumers about the appropriate method of cooking of rice to prevent the loss of these highly essential micronutrients. The selected method if adopted will improve the iron and zinc status of the populations.

2. Materials and methods

2.1. Terminology

The terminology used in the text has been defined in the following manners

1. *Fortified rice* refers to the polished rice obtained after dehusking and milling of iron & zinc fortified paddy grains. Paddy grains were fortified through parboiling process.
2. *Non-fortified rice* means the normal polished rice obtained after dehusking and milling of natural paddy grains. It aims to differentiate normal polished rice from fortified rice.
3. *Cooking in excess water* implies cooking of rice in excess water in an open vessel and draining off the residual water when cooking is over.
4. *Milled rice/polished rice* means the white rice obtained after 120 s polishing of brown rice of any variety.

2.2. Materials

A total of six rice varieties, out of which four rice varieties namely Sampada, DRR Dhan 44, DRR Dhan 45, RP Bio 226 developed and released by ICAR-Indian Institute of Rice Research, and two popular varieties MTU 1010, BPT 5204 from Acharya N. G. Ranga Agricultural University, were included in this study. They were grown in field plots with the same soil type. Rice grains were harvested at maturity, with a great care to minimize the chance of soil contamination in the field.

2.3. Iron and zinc fortification

Iron and Zinc fortification of paddy was done with slightly modified method of Prom-U-Thai et al. (2011). In short, dried 500 g lots of paddy were soaked overnight (14 h) in 1000 ml of the mixed NaFeEDTA: $ZnSO_4$ solution containing 200: 150 mg/l at pH ~ 3.5. Next day, the mixture was boiled for 10 min. After cooling, excess solution was drained off and the remaining paddy was steamed at 120 °C for ten minutes using pressure cooker. The fortified paddy grains were cooled and sun dried (~10% moisture).

2.4. Husking and milling

The dried paddy grains (500 g) of both fortified and non-fortified were separated into brown rice and husk with a non-ferrous dehusker (Lab Rice Huller H-750, Krishi International, India). For each sample, 200 g of the brown rice were milled for 120 s to yield white rice by using a non-ferrous milling machine (Lab Rice Polisher M-780, Krishi International, India). Non-ferrous materials were used in dehusker and polishing machine to minimize Fe contamination in rice samples. White rice, also known as polished or milled rice, were cleaned, made free of dust, dirt and foreign materials and packed in air tight containers at room temperature prior to cooking.

2.5. Cooking methods

The polished rice samples both of fortified and non-fortified (100 g each) were subjected to five different types of cooking methods (Table 1) namely, 1) Cooking in rice cooker without prior washing (NRC); 2) Cooking in rice cooker with prior washing (WRC); 3) Cooking in excess water in open vessel without prior washing (NRE); 4) Cooking in excess water in open vessel with prior washing (WRE); and 5) Cooking in excess water in open vessel with prior washing and soaking (WRSE). Throughout the experiments, distilled water was used where ever water was required. In washing step, rice grains were washed with 2.5 × 3 times of water. For cooking in rice cooker, the ratio of rice grain and water was 1:2.5 w/v while for cooking in excess water, the ratio of rice grain and water was 1: 6 w/v. All experiments were carried out in triplicate.

2.6. Determination of iron and zinc content

All the cooked rice samples were dried in hot air oven at 55–60 °C and the moisture content was kept around 10%. Un-cooked polished rice samples of all the six rice varieties were also dried to maintain moisture content at around 10%. Sample moisture was monitored using Moisture Meter (Model, MB400, Citizen India Ltd). Head rice grains of the test samples were picked up, cleaned thoroughly with tissue papers to remove minute fine dust particles, and packed in air tight containers at room temperature. Iron and zinc content were estimated using standardized non-destructive "Energy Dispersive X-Ray Fluorescence Spectrophotometry (ED-XRF; Model, Oxford Instrument X Supreme 8000) method (Azam et al., 2017a). The obtained iron and zinc values were expressed in ppm (parts per million). The loss in micronutrients (iron/zinc) due to washing/cooking has been expressed as percentage which has been calculated using the following formulae

$$\%\text{Loss} = (X - Y)/X * 100$$

Where,

X = Micronutrient content (ppm) in dry uncooked rice
Y = Micronutrient content (ppm) in dry cooked rice

2.7. Statistical analysis

The data for estimates of Fe and Zn recorded for different cooking treatments in non-fortified and fortified rice were treated as split plot design and subjected to analysis of variance (ANOVA) to determine cooking treatment effects (main effects) and varietal effect (sub effects). The Fisher's least significant difference test at $P < 0.05$ was used to compare the means of each cooking treatment effects. All the statistical analysis was carried using program PROC GLM SAS and also statistical significant differences ($P < 0.05$) in different cooking methods were also graphically represented by diffograms using statistical software SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

The analysis of variance showed significant variation ($P < 0.001$) in the concentration of micronutrients among the cooking treatments; varieties and interaction between the two. Iron content of test samples is shown in Tables 2 and 3 while their zinc content has been shown in Tables 4 and 5. In uncooked fortified polished rice (UPR), the average Fe content was 106.31 ± 12.56 ppm with a range from 93.10 ± 12.34 ppm in DRR Dhan 45 to 124.13 ± 4.5 ppm in RP Bio 226. There were significant differences among the varieties (LSD = 10.2; $P < 0.05$).

3.1. Cooking effect on iron fortified rice

The average iron content in the fortified rice was 104.89 ± 10.08 ppm ranging from 92.4 ± 6.26 ppm (DRR Dhan 45) to 123.3 ± 3.48 (RP Bio 226), and their varietal means were significantly different (LSD = 5.89; $P < 0.05$). The average Fe content in WRC treated rice, was 82.13 ± 11.87 ppm ranging from 65.7 ± 7.66 ppm (DRR Dhan 45) to 91.1 ± 19.54 ppm (Sampada). It showed significant difference (LSD = 17.33; $P < 0.05$) in varietal means. After NRE treatment, the Fe content varied from 40.37 ± 2.75 ppm (DRR Dhan 45) to 55.0 ± 2.23 ppm (RP Bio 226) with an average of 47.56 ± 5.49 ppm. The LSD value (3.28, $P < 0.05$) showed that the varietal means were significantly different. The variation in iron

Table 1
Description of cooking methods.

Cooking methods	Description of methods
1) Cooking in rice cooker without previous washing (NRC)	100 g of cleaned polished rice obtained after hulling and milling of paddy seeds, were taken in a rice cooker and 250 ml of distilled water was added. Power of the rice cooker was switched on. When cooking completed, the power of cooker automatically switched off. It was allowed to cool for 30 min. The cooked grain absorbed all water.
2) Cooking in rice cooker with previous washing (WRC)	100 g of cleaned polished rice obtained after hulling and milling of paddy seeds were washed with 250 ml of water. Washing was repeated thrice. The total volume of water for washing was 750 ml. Water was completely drained off and rinsed grains were put in the cooker and 250 ml water was added and rice cooker was switched on. When cooking completed, the power of cooker automatically switched off. It was allowed to cool for 30 min. The cooked grain absorbed all water.
3) Cooking in excess water in open vessel without previous washing (NRE)	100 g of cleaned polished rice obtained after hulling and milling of paddy seeds, were directly taken in a vessel (commonly used for rice cooking in home) and 600 ml was added to it. Rice was cooked on hot plate with temperature set at 380 °C and the excess water was completely drained off when the rice was fully cooked.
4) Cooking in excess water in open vessel with previous washing (WRE)	100 g of cleaned polished rice obtained after hulling and milling of paddy seeds were washed with 250 ml of water. Washing was repeated thrice with same volume of water. The total volume of water used for washing was 750 ml. Water was completely drained off and rinsed grains were taken in the vessel and 600 ml water was added. Rice was cooked on hot plate with temperature set at 380 °C and the excess water was completely drained off when the rice was fully cooked.
5) Cooking in excess water in open vessel with previous washing and soaking (WRSE)	100 g of cleaned polished rice obtained after hulling and milling of paddy seeds were washed with 250 ml of water. Washing was repeated thrice with same volume of water. After 3 times washing, rice grains were soaked in 250 ml water. After half an hour, 350 ml water was added to it and cooked on hot plate with temperature set at 380 °C and the excess water was completely drained off when the rice was fully cooked.

Table 2
Mean Iron content (parts per million) in uncooked and cooked rice samples of fortified rice.

Treatment	BPT 5204	DRR Dhan 44	DRR Dhan 45	MTU 1010	RP Bio 226	Sampada	TRT Mean
UPR	103.67 ± 12.59^{a}	104.17 ± 3.29^{a}	93.10 ± 12.34^{a}	104.27 ± 9.28^{a}	124.13 ± 4.5^{a}	108.5 ± 4.97^{a}	106.31 ± 12.56^{A}
NRC	99.53 ± 1.84^{a} (3.99)	103.5 ± 1.67^{a} 0.64)	92.4 ± 6.26^{a} (0.75)	103.90 ± 1.56^{a} (0.35)	123.2 ± 3.48^{a} (0.75)	107.8 ± 2.23^{a} (0.65)	104.89 ± 10.08^{A} (1.34)
WRC	79.67 ± 7.71^{b} (23.15)	89.37 ± 4.07^{b} (14.21)	65.7 ± 7.66^{b} (29.43)	82.47 ± 4.31^{b} (20.91)	84.5 ± 6.40^{b} (31.93)	91.1 ± 19.54^{b} (16.04)	82.13 ± 11.87^{B} (22.74)
NRE	44.63 ± 1.47^{c} (56.95)	44.93 ± 2.40^{c} (56.87)	40.37 ± 2.75^{c} (56.64)	55 ± 2.23^{c} (47.25)	47.67 ± 2.1^{c} (61.6)	52.77 ± 2.6^{c} (51.36)	47.56 ± 5.49^{C} (55.26)
WRE	39.57 ± 0.83^{c} (61.83)	41.2 ± 3.14^{c} (60.45)	34 ± 4.40^{c} (63.48)	53.83 ± 5.82^{c} (48.37)	44.17 ± 3.35^{c} (64.42)	50.4 ± 4.01^{c} (53.55)	43.86 ± 7.58^{D} (58.74)
WRSE	38.57 ± 0.40^{c} (62.8)	40.83 ± 1.66^{c} (60.8)	34.73 ± 2.2^{c} (62.7)	53.1 ± 2.63^{c} (49.07)	43.9 ± 1.35^{c} (64.63)	50.1 ± 4.37^{c} (53.82)	43.54 ± 6.88^{D} (59.04)
Mean	67.61	70.67	60.05	75.26	77.93	76.78	71.38
p-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	–
CV (%)	5.2	3.59	7.06	4.27	4.51	9.65	–
SE(d)	2.87	2.07	3.46	2.62	2.87	6.05	–
LSD at 5%	6.39	4.61	7.71	5.84	6.38	13.48	–

TRT- Treatment; UPR-Uncooked polished rice; NRC-Cooking in rice cooker without previous washing; WRC-Cooking in rice cooker with previous washing; NRE-Cooking in excess water in open vessel without previous washing; WRE-Cooking in excess water in open vessel with previous washing; and WRSE-Cooking in excess water in open vessel with previous washing and soaking; Figures in parenthesis represent percent loss of Iron content after different methods of cooking; values followed by different letters within the same treatment under different varieties are significantly different ($p < 0.05$).

Table 3
Mean Iron content (parts per million) in uncooked and cooked rice samples of non-fortified rice.

Treatment	BPT 5204	DRR Dhan 44	DRR Dhan 45	MTU 1010	RP Bio 226	Sampada	TRT Mean
UPR	8.5 ± 0.30^{a}	8.5 ± 0.26^{a}	6.02 ± 0.17^{a}	6.3 ± 0.36^{a}	8 ± 0.36^{a}	7.3 ± 0.20^{a}	7.44 ± 1.05^{A}
NRC	8.43 ± 0.12^{a} (0.82)	8.43 ± 0.12^{a} (0.82)	6.01 ± 0.06^{a} (0.17)	6.17 ± 0.06^{a} (2.06)	7.97 ± 0.15^{a} (0.38)	7.17 ± 0.23^{a} (1.78)	7.36 ± 1.03^{A} (1.08)
WRC	6.5 ± 0.17^{b} (23.53)	3.9 ± 0.26^{b} (54.12)	5.2 ± 0.30^{b} (13.62)	5.5 ± 0.17^{b} (12.7)	5.8 ± 0.2^{b} (27.5)	5 ± 0.36^{b} (31.51)	5.32 ± 0.84^{B} (28.49)
NRE	5.57 ± 0.32^{c} (34.47)	3.27 ± 0.21^{c} (61.53)	4.13 ± 0.15^{c} (31.4)	5.23 ± 0.25^{bc} (16.98)	5.43 ± 0.12^{bc} (32.13)	4.3 ± 0.17^{c} (41.1)	4.66 ± 0.87^{C} (37.37)
WRE	5.2 ± 0.36^{c} (38.82)	2.9 ± 0.40^{c} (65.88)	3.7 ± 0.20^{d} (38.54)	4.9 ± 0.44 ^{cd} (22.22)	5.3 ± 0.26^{c} (33.75)	4.2 ± 0.30^{c} (42.47)	4.37 ± 0.93^{D} (41.26)
WRSE	4.93 ± 0.59^{c} (42.0)	2.83 ± 0.21^{c} (66.71)	3.63 ± 0.06^{d} (39.7)	4.8 ± 0.26^{d} (23.81)	5.17 ± 0.15^{c} (35.38)	4.07 ± 0.06^{c} (44.25)	4.24 ± 0.87^{D} (43.01)
Mean	6.52	4.97	4.78	5.48	6.28	5.34	5.56
p-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	–
CV (%)	5.54	5.64	3.37	4.32	3.61	4.93	–
SE(d)	0.3	0.23	0.13	0.19	0.18	0.21	–
LSD at 5%	0.65	0.50	0.29	0.43	0.41	0.47	–

TRT- Treatment; UPR-Uncooked polished rice; NRC-Cooking in rice cooker without previous washing; WRC-Cooking in rice cooker with previous washing; NRE-Cooking in excess water in open vessel without previous washing; WRE-Cooking in excess water in open vessel with previous washing; and WRSE-Cooking in excess water in open vessel with previous washing and soaking; Figures in parenthesis represent percent loss of Iron content after different methods of cooking; values followed by different letters within the same treatment under different varieties are significantly different ($p < 0.05$).

Table 4
Mean Zinc content (parts per million) in uncooked and cooked rice samples of fortified rice.

Treatment	BPT 5204	DRR Dhan 44	DRR Dhan 45	MTU 1010	RP Bio 226	Sampada	TRT Mean
UPR	90.50 ± 7.38^{a}	89.9 ± 3.50^{a}	93.33 ± 3.96^{a}	97.6 ± 2.42^{a}	115.53 ± 3.96^{a}	99.47 ± 3.38^{a}	97.72 ± 9.75^{A}
NRC	89.03 ± 3.45^{a} (1.62)	88.97 ± 2.52^{a} (1.03)	92.73 ± 1.64^{a} (0.64)	96.60 ± 0.87^{a} (1.02)	114.17 ± 2.94^{a} (1.18)	97.67 ± 1.64^{a} (1.81)	96.53 ± 9.26^{A} (1.22)
WRC	76.9 ± 5.54^{b} (15.03)	77.53 ± 7.71^{b} (13.76)	75.63 ± 6.19^{b} (18.96)	78.5 ± 4.62^{b} (19.57)	91.27 ± 0.06^{b} (21)	81.77 ± 14.51^{b} (17.79)	80.27 ± 8.47^{B} (17.86)
NRE	36.37 ± 1.96^{c} (59.81)	38.87 ± 1.1^{c} (56.76)	36.57 ± 2.12^{c} (60.82)	43.83 ± 1.27^{c} (55.09)	45.63 ± 1.17^{c} (60.5)	40.1 ± 1.76^{c} (59.69)	40.23 ± 3.82^{C} (58.83)
WRE	33.5 ± 1.83^{c} (62.98)	35.63 ± 2.77^{c} (60.37)	34.33 ± 3.01^{c} (63.22)	40.93 ± 1.96^{c} (58.06)	39.43 ± 8.02^{d} (65.87)	37.67 ± 2.78^{c} (62.13)	36.92 ± 4.35^{D} (62.22)
WRSE	33.43 ± 0.59^{c} (63.06)	35.5 ± 1.23^{c} (60.51)	33.93 ± 0.81^{c} (63.65)	40.97 ± 1.19^{c} (58.02)	39.3 ± 3.21^{d} (65.98)	37.07 ± 1.14^{c} (62.73)	36.70 ± 3.12^{D} (62.44)
Mean	59.96	61.07	61.09	66.41	74.22	65.62	64.72
p-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	–
CV (%)	5.6	6.8	5.83	3.66	4.33	9.16	–
SE(d)	2.74	3.39	2.91	1.98	2.62	4.91	–
LSD at 5%	6.1	7.55	6.47	4.41	5.84	10.93	–

TRT- Treatment; UPR-Uncooked polished rice; NRC-Cooking in rice cooker without previous washing; WRC-Cooking in rice cooker with previous washing; NRE-Cooking in excess water in open vessel without previous washing; WRE-Cooking in excess water in open vessel with previous washing; and WRSE-Cooking in excess water in open vessel with previous washing and soaking; Figures in parenthesis represent percent loss of Zinc content after different methods of cooking; values followed by different letters within the same treatment under different varieties are significantly different ($p < 0.05$).

content after WRE treatment, ranged from 34 ± 4.40 ppm (DRR Dhan 45) to 53.83 ± 5.82 ppm (MTU 1010) with an average of 43.86 ± 6.88 ppm and significant varietal means differences (LSD = 5.16; P < 0.05). In WRSE treated rice, Fe content ranged from 34.73 ± 2.2 ppm (DRR Dhan 45) to 53.1 ± 2.63 ppm (MTU 1010) with an average of 43.54 ± 6.88 ppm and significant varietal (LSD = 4.01; P < 0.05). Varietal effects for different cooking treatments on Fe in fortified rice were significant. However, all

Table 5
Mean Zinc content (parts per million) in uncooked and cooked rice samples of non-fortified rice.

Treatment	BPT 5204	DRR Dhan 44	DRR Dhan 45	MTU 1010	RP Bio 226	Sampada	TRT Mean
UPR	11.5 ± 0.36^{a}	12.3 ± 0.7^{a}	19.9 ± 0.46^{a}	14.57 ± 0.31^{a}	16.63 ± 0.29^{a}	13.53 ± 0.32^{a}	14.74 ± 2.94^{A}
NRC	11.10 ± 0.21^{a} (3.48)	12.10 ± 0.6^{a} (1.63)	19.63 ± 0.42^{a} (1.36)	14.17 ± 0.06^{a} (2.75)	16 ± 0.36^{a} (3.79)	13.23 ± 0.25^{a} (2.22)	14.57 ± 2.88^{A} (1.15)
WRC	9.6 ± 0.46^{b} (16.52)	10.4 ± 0.40^{b} (15.45)	16.7 ± 0.36^{b} (16.08)	12.6 ± 0.44^{b} (13.52)	13.93 ± 0.31^{b} (16.24)	10 ± 0.46^{b} (26.09)	12.21 ± 2.62^{B} (17.16)
NRE	6.8 ± 0.26^{c} (37.39)	8.93 ± 0.42^{c} (25.77)	13.33 ± 0.42^{c} (31.66)	9.33 ± 0.31^{c} (33.22)	11.37 ± 0.47^{c} (27.84)	8.2 ± 0.35^{c} (37.18)	9.66 ± 2.22^{C} (33.31)
WRE	6.6 ± 0.06^{c} (42.61)	8.97 ± 0.42^{c} (27.07)	13 ± 0.36^{c} (34.67)	9.5 ± 0.44^{c} (34.8)	10.8 ± 0.20^{c} (35.06)	8.2 ± 0.30^{c} (39.39)	9.51 ± 2.09^{CD} (35.48)
WRSE	6.4 ± 0.40^{c} (44.35)	8.7 ± 0.17^{c} (29.27)	12.97 ± 0.97^{c} (34.82)	9.37 ± 0.21^{c} (35.69)	10.2 ± 0.3^{d} (38.67)	8.17 ± .29^{c} (39.62)	9.3 ± 2.11^{D} (36.91)
Mean	8.72	10.24	15.92	11.64	13.25	10.22	11.66
p-Value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	–
CV (%)	4.09	2.51	3.02	2.59	2.45	2.95	–
SE(d)	0.29	0.21	0.39	0.25	0.27	0.25	–
LSD at 5%	0.6488	0.467	0.8754	0.5483	0.5914	0.5485	–

TRT- Treatment; UPR-Uncooked polished rice; NRC-Cooking in rice cooker without previous washing; WRC-Cooking in rice cooker with previous washing; NRE-Cooking in excess water in open vessel without previous washing; WRE-Cooking in excess water in open vessel with previous washing; and WRSE-Cooking in excess water in open vessel with previous washing and soaking; Figures in parenthesis represent percent loss of Zinc content after different methods of cooking; values followed by different letters within the same treatment under different varieties are significantly different ($p < 0.05$).

the varieties showed similar trend in the loss of Fe content. Fe content in fortified rice remained unchanged under unpolished rice (UPR) and cooking rice in rice cooker without prior washing (NRC) for all the varieties. Irrespective of varieties, under cooking rice with prior washing (WRC) Fe content was significantly differed ($p < 0.05$) from all other cooking methods.

3.2. Cooking effect of iron nonfortified rice

The uncooked polished rice (UPR) of six varieties contains an average of 7.44 ± 1.05 ppm of Fe in non-fortified rice ranging from 6.02 ± 0.17 ppm (DRR Dhan 45) to 8.5 ppm (BPT 5204, DRR Dhan 44) (Table 3). Significant differences (LSD = 0.50 at $P < 0.05$) were observed among the varieties. After NRC treatment, the average Fe content in non-fortified cooked rice was 7.36 ± 1.03 ppm ranging from 6.01 ± 0.06 ppm (DRR Dhan 45) to 8.43 ± 0.12 ppm (BPT 5204, DRR Dhan 44). Similarly, the average Fe contents in non-fortified rice were 5.32 ± 0.84, 4.66 ± 0.87, 4.37 ± 0.93 and 4.24 ± 0.87 ppm in WRC, NRE, WRE and WRSE, respectively (Table 3). The varietal means differed significantly in each of the cooking treatments. Varietal effects for different cooking treatments on Fe content in non-fortified rice were significant which is evident from the LSD values at $P < 0.05$ (0.45, WRC; 0.39, NRE; 0.62, WRE; and 0.45, WRSE).

Diffogram studies also reflected significant differences in different cooking methods for iron and zinc content in fortified and non-fortified rice (Figs. 1 and 2). In non-fortified rice, the iron content of UPR registered significant difference with all other cooking methods except NRC. Similarly, WRC recorded significantly with WRSE, WRE and NRC except NRE. NRE was non-significant difference with WRE and WRSE between WRE and WRSE also recorded non-significant difference. In fortified rice also Iron content showed similar trend as that of non-fortified rice (Fig. 1).

3.3. Cooking effect on zinc in fortified rice

Zinc content in uncooked fortified polished rice (UPR) among six varieties ranged from 115.53 ± 3.96 ppm in RP Bio 226 to 89.9 ± 3.50 ppm in DDR Dhan 44 which yielded an average of 97.72 ± 9.75 ppm. Their varietal means were significantly different (LSD = 6.43; $P < 0.05$). Average zinc content under NRC treated fortified rice was 96.53 ± 9.26 ppm ranging from 88.97 ± 2.52 ppm (DRR Dhan 45) to 114.17 ± 2.94 ppm (RP Bio 226) and showed significant difference in varietal means (LSD = 3.66; $P < 0.05$). Similarly, the average Zn contents in fortified rice of these six varieties were 80.27 ± 8.47, 40.23 ± 3.82, 36.92 ± 4.35 and 36.70 ± 3.12 ppm after the treatment of WRC, NRE, WRE, and WRSE, respectively (Table 4). The varietal means for Zn content differed significantly in each of the cooking treatments which is evident from the LSD at $P < 0.05$ (14.64, WRC; 2.91, NRE; 7.16, WRE; and 3.12, WRSE). Varietal effects for different cooking treatments on Zn in fortified rice were significant. The effects of UPR and NRC on Zn content in fortified rice were similar for all the varieties. Irrespective of varieties, WRC effect on Zn content was significantly different ($p < 0.05$) from all the cooking methods. However, in RP Bio 226, cooking treatments WRE and WRSE effects on zinc content were significantly different ($P < 0.05$).

3.4. Cooking effect on zinc in non-fortified rice

Zinc content in uncooked non-fortified polished rice (UPR) among the six varieties ranged from 11.5 ± 0.36 ppm (BPT 5204) to 19.9 ± 0.46 ppm (DDR Dhan 45) which showed an average of 14.74 ± 2.94 ppm and the varietal means were significantly different (LSD = 0.76; $P < 0.05$). The average zinc content in NRC treated rice was 14.57 ± 2.88 ppm ranging from 11.1 ± 0.21 ppm (BPT 5204) to 19.63 ± 0.42 ppm (DRR Dhan 45) and the varietal means were significantly different (LSD = 0.42; $P < 0.05$). Similarly, the average Zn contents in the non-fortified rice of these six varieties were 12.21 ± 2.62 ppm, 9.66 ± 2.22 ppm, 9.51 ± 2.04 ppm, and 9.3 ± 2.11 ppm in WRC, NRE, WRE and WRSE, respectively (Table 5). In these treatments, the varietal means of zinc content differed significantly as indicated by the LSD values at $P < 0.05$ (0.76, WRC; 0.42, NRE; 0.66, WRE; and 0.93, WRSE). The varietal effects for different cooking treatments on zinc content in non-fortified rice were similar to the fortified rice.

In non-fortified rice, the zinc content of UPR showed similar trend as that of iron content, where it recorded significant difference with all other cooking methods except NRC. WRC showed significant difference with NRE, WRE and WRSE cooking methods. NRE showed non-significant difference with WRE and WRSE. The zinc contents of WRE and WRSE were non significantly different as that of iron content in non-fortified rice. In fortified rice, zinc content of UPR showed similar result as that of non-fortified rice. The NRE showed non-significant difference compared to WRE and WRSE for zinc content (Fig. 2).

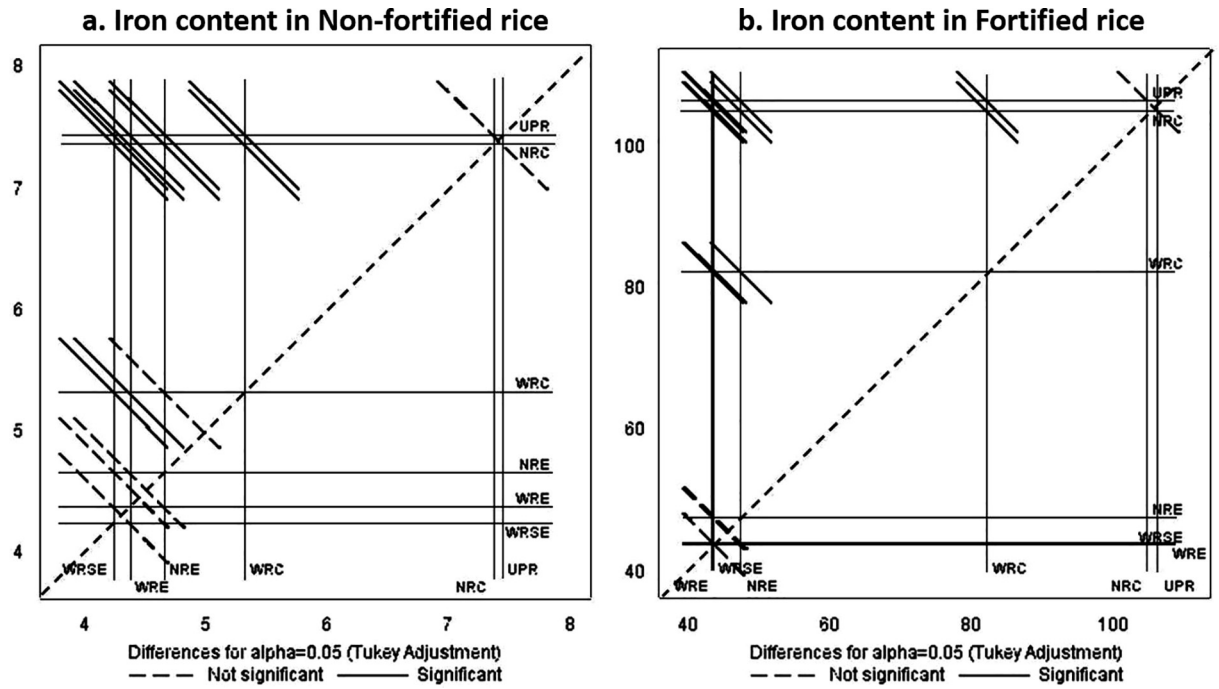

Fig. 1. Diffograms showing significant differences in iron content of (a) Non-fortified rice and (b) Fortified rice in different cooking methods. Bold horizontal and vertical lines represent the different cooking methods. The small lines passing through the squares diagonally represent non-significant or significant differences between the two corresponding cooking methods. UPR-Uncooked polished rice; NRC-Cooking in rice cooker without prior washing; WRC-Cooking in rice cooker with prior washing; NRE-Cooking in excess water in open vessel without prior washing; WRE-Cooking in excess water in open vessel with prior washing; and WRSE-Cooking in excess water in open vessel with prior washing and soaking.

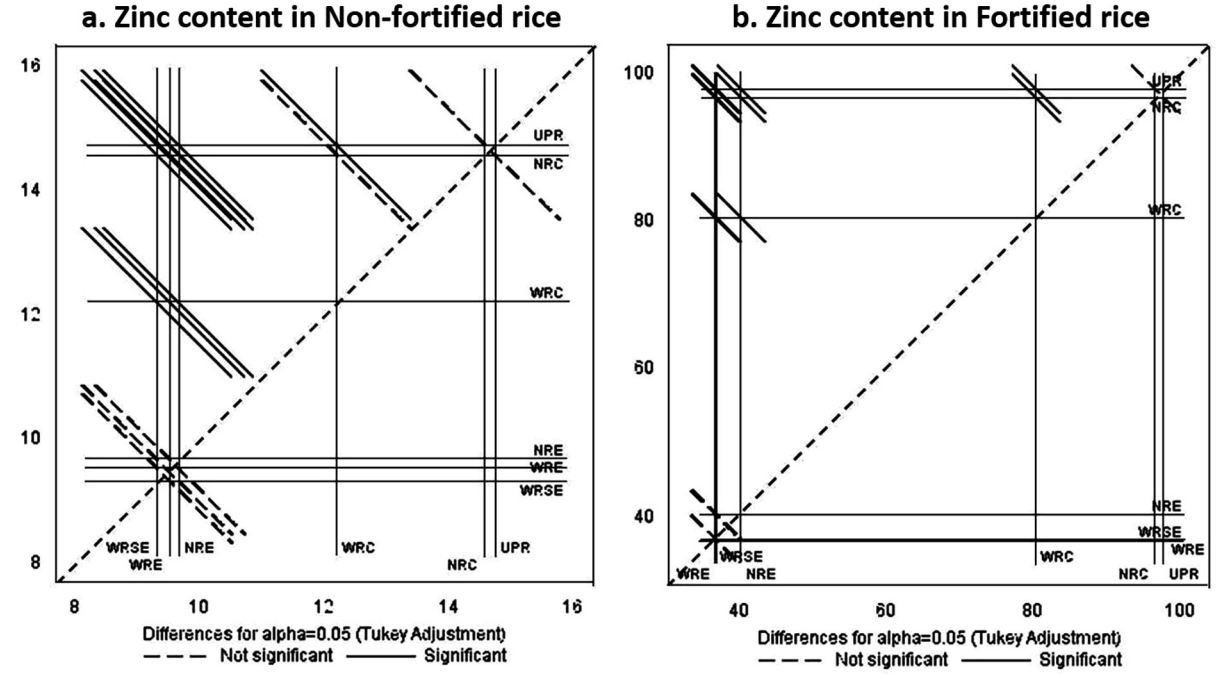

Fig. 2. Diffograms showing significant differences in zinc content of (a) Non-fortified rice and (b) Fortified rice in different cooking methods. Bold horizontal and vertical lines represent the different cooking methods. The small lines passing through the squares diagonally represent non-significant or significant differences between the two corresponding cooking methods. UPR-Uncooked polished rice; NRC-Cooking in rice cooker without prior washing; WRC-Cooking in rice cooker with prior washing; NRE-Cooking in excess water in open vessel without prior washing; WRE-Cooking in excess water in open vessel with prior washing; and WRSE-Cooking in excess water in open vessel with prior washing and soaking.

3.5. Cooking effects on iron and zinc content

Cooking rice in rice cooker without previous washing (NRC) did not cause significant loss in iron and zinc content. The Fe losses in the samples were very low ranging from 0.17% to 1.78% and 0.35% to 3.99% in non-fortified and fortified rice, respectively. While the average Zn loss in non-fortified and fortified rice was only 1.15% and 1.22%, respectively. The negligible loss of iron and zinc after this treatment can be attributed to the fact that heat during cooking does not have any affect on Fe and Zn content.

Cooking in rice cooker after washing (WRC) brought a significant loss of iron and zinc content in both non-fortified (iron, 28.49%; Zn, 17.16%) and fortified rice (iron, 22.74%; Zn, 17.86%). Iron and zinc content in WRC treated rice was significantly different ($P < 0.05$) from those of NRC and UPR. This high percentage loss of iron and zinc both in non-fortified and fortified rice is due to the leaching of minerals during washing of the rice before cooking.

The NRE treatment involving cooking of rice in excess water without washing, resulted in high loss in iron and zinc content in both non-fortified (Fe, 37.37%; Zn, 33.31%) and fortified rice (Fe, 55.26%; Zn, 58.83%) over WRC and NRC. The Fe content did not vary among varieties and there was no significant differences observed for varieties in fortified rice. In non-fortified rice also, NRE treatment showed similar result where no significant difference was observed for varieties BPT 5204, DRR Dhan 44, DRR Dhan 45 and Sampada compared to MTU 1010 and RP Bio 226.

When rice samples were washed and subsequently cooked in excess water (WRE treatment), further loss in iron and zinc was observed. The average iron loss in non-fortified and fortified rice increased to 41.26% (Table 3) and 58.74% (Table 2), respectively. Similarly, zinc loss in fortified and non-fortified rice increased to 62.22% (Table 5) and 35.48% (Table 4), respectively. In WRE treatment, there was no significant difference observed among all the six varieties for iron content in fortified rice. Whereas in non-fortified rice, out of six varieties BPT 5204, DRR Dhan 44, RP Bio 226 and Sampada were significantly different ($p < 0.05$) compared to DRR Dhan 45 and MTU 1010. Zinc content also showed similar trend as that of iron content in non-fortified and fortified rice. Non-fortified rice of RP Bio 226, was significantly deviated from other varieties.

Cooking rice in excess water in open vessel with prior washing and soaking (WRSE) further loss of iron and zinc was observed among non-fortified and fortified rice. An average loss of 43.01% and 59.04% of iron was recorded in non-fortified and fortified rice, respectively and the corresponding zinc loss was 36.91% and 62.24%. Although maximum loss of iron and zinc occurred in WRSE treatment but Data shows (Tables 2–5) that WRSE treatment did not bring significant change over NRE or WRE with few exceptions.

4. Discussion

On fortification there was 13.3 times increase of Fe content and 5.6 times increase in Zn content among 6 varieties in the study. Fortification of the variety DRR Dhan 45 with lowest Fe content (6.02 ± 0.17 ppm) has increased the iron level by 14.5 folds, while in BPT 5204 and DRR Dhan 44, both having highest Fe in baseline, the increase was 11.2 folds. Similarly, fortification of low Zn containing variety, BPT 5204 increased the zinc level by 6.9 folds, while in variety DRR Dhan 45 with highest Zn among 6 varieties, the rise was 3.7 folds. Interestingly, in RP Bio 226 and MTU 1010, 5.7 folds of increase in zinc level was observed. The varietal effect was significant for the fortification of Fe and Zn. Nevertheless, cooking treatment effected significantly in retention of the concentration of Fe and Zn in different varieties.

The method used for fortification is an intruding factor for amount of micronutrient content. Mixing nutrient- premix with normal polished rice in the ratio of 1:100 reduces iron content due to washing Rosales (2010). The washing of the fortified rice only for 90 s and subsequent cooking in a rice cooker caused very high loss of iron (47%). This kind of fortified rice may not be suitable for cooking in excess water. In the present study, the incurred loss of minerals due to washing of fortified rice was comparatively low ranging from 14.21% to 31.93% and 13.76% to 21.00% of iron and zinc, respectively, which showed that the minerals were absorbed in the endosperm of the grains due to the method of fortification adopted in this study.

Wieringa et al. (2014) fortified normal polished rice by mixing with rice pre-mix and examined the effects of different cooking methods on retention of iron and zinc and other nutrients in cooked rice. When fortified rice samples were cooked in "excess water + soaking", "excess water" and "boiling" methods, the overall retention of iron and zinc was 106.5 ± 51.3%, 101.5 ± 48.1%, 114. 8 ± 45.1%, and 92.3 ± 15.3%, 85.7 ± 19.2, 95.0 ± 7.5%, respectively. These values indicate that in most of the cooked samples, retention of iron and zinc was more than 100%. Retention of iron and zinc more than 100% in the cooked rice seems to be unrealistic and no explanation was offered by the authors. It may either be due to contamination during cooking or experimental error or due to uneven distribution of micronutrients in the samples. In the above study, the rice premixes used for making fortified rice were produced by the expensive methods of wax coating, cold extrusion with a binding agent and hot extrusion. The initial investment cost for production of such fortified rice is very high. High investment cost has been identified as one of the major constraints to scaling up rice fortification (Piccoli et al., 2012). On the other hand, fortification of rice with iron and zinc through parboiling process adopted in the present study is simple, cost-effective and economically viable and more attractive than other methods and can be carried out with the existing parboiling facilities.

Among the cooking methods used in this study, minimum loss of iron and zinc was observed in NRC treatment. However, this is not a preferred method as rice is cooked without washing of the grains. The methods "washing and subsequent cooking in a rice cooker/pressure cooker (WRC)" and "cooking in excess water (NRE/WRE/WRSE)" are popular in India. The loss of iron and zinc in the WRC method is only due to washing of the rice before cooking.

Cooking in excess water with additional steps of washing and soaking resulted in higher iron & zinc losses in non-fortified rice (iron-37.37%, zinc-33.31% NRE; iron-41.26%, zinc-35.48%, WRE; iron-43.01%, zinc-36.91, WRSE) as well as in fortified rice (iron-55.26%, zinc-58.83%, NRE; iron-58.74%, zinc-62.22%, WRE; iron-59.04%, zinc-62.44%, WRSE). These losses can be attributed to the fact that some amount of iron and zinc had leached in water during washing and then in soaking steps. Further leaching of iron and zinc took place when rice was cooked in excess water and residual water was drained off. Because of the combined effects of these steps, maximum loss of iron was observed in the case of WRSE treatment. Suman and Boora (2015) cooked normal polished rice of different varieties with pressure, microwave and solar cooking methods after washing and soaking the samples. The authors reported loss of iron ranging from 23.2% to 31.2% and zinc from 12.5 to 15.3%.

The results also revealed that when rice was cooked in excess water under different cooking conditions, higher loss of iron (55.26% to 59.09%, Table 3) and zinc (58.83% to 62.44%, Table 5) was observed in fortified rice in comparison to the non-fortified rice (iron, 37.37% to 43.01%, Table 2; zinc, 33.31% to 36.91%, Table 4). The trends of mineral losses can be attributed to the fact that iron and zinc are loosely bound in grains of fortified rice which

upon cooking in excess of water get leached into water and are lost when excess water is drained off (Rosales (2010).

When cooking methods were compared for iron and zinc retention in fortified as well as in non-fortified rice of a variety; observed differences were statistically non-significant (Tables 2 and 3). However, the Fe content in non-fortified rice DRR Dhan 45 and MTU-1010 reduced due to WRE and WRSE. The Fe content in UPR of non-fortified rice was significantly higher than all cooking method except NRC. However palatability of unpolished rice is not possible and therefore cooking method such as NRC is better option to reduce the loss of Fe and Zn under cooking. In WRC method Fe content in non-fortified was significantly different ($p < 0.05$) from WSRE, WRE and UPR but not with NRE method (Fig. 1a). Cooking methods affected the Fe content of fortified rice. WRC significantly ($p < 0.05$) differed from WRE, WRSE, NRE and NRC, while WRE, WRSE and NRE did not differ significantly (Fig. 1b). The effect of cooking methods on retention of iron and zinc is variety independent with few exceptions. The genotypic variation for grain type, chemical constitution and cropping ecosystem do not influence the reaction of varieties to different cooking treatments (Trijatmiko et al. 2016). Mwale et al. (2018) cooked normal polished rice in excess water (1:6) and residual water was discarded after completion of cooking. The reported average loss of iron (8.2%) and zinc (7.7%) was unexpectedly very low.

Cooking treatments of non-fortified and fortified rice were not significantly different but varietal responses to the cooking methods were non-significant (Tables 2–4). Except in RP Bio 226, significant loss of Zn occurred in all varieties when fortified rice was cooked by WRE method. UPR unfortified rice differed for Zn content with all the cooking methods except NRC. WRC differed significantly from WRSE, WRE and NRE, while WRSE, WRE and NRE showed similar effects on Zn content (Fig. 2a). Similarly, for fortified rice, UPR differed significantly from all the cooking method except NRC. WRC effect was different from WRE, WRSE and NRE, while WRE, WRSE and NRE effect on Zn did not differ from each other (Fig. 2b). Iron content in uncooked unfortified polished rice reported by Khatoon and Prakash (2006) ranged from 15.0 ± 0.38 ppm to 19.0 ± 0.003 ppm which was higher than the iron content recorded in the present study (6.02 ± 0.17 ppm to 8.5 ± 0.30 ppm). The iron loss due to washing of normal polished grains before cooking was 33–50% which was higher than the findings of the present study (MTU 1010, 12.70% to Sampada, 31.51%) with an exception of DRR Dhan 44 (54.12%). Poor iron content (~2 ppm) in polished grains has also been reported by other researchers (Bouis et al., 2011; Trijatmiko et al., 2006). Intrestingly, Ebuehi and Oyewole (2007) did not find any significant loss in iron content, when rice was cooked in a rice cooker after washing. A contrasting results was reported by Kimura and Itokawa (1990) that the content of several minerals including iron increased over the uncooked rice (100–300%) when normal polished rice was cooked after washing and soaking. The author implied that the higher retention (>100%) of minerals may have been caused by contamination of water or pan used for cooking.

Although the cooking of fortified rice in excess water under different conditions (NRE, WRE and WRSE) brought very high percentage of loss of iron (55.26% to 59.04%) and zinc (58.83% to 62.44%), the retained amount of iron (47.56 ± 5.49 ppm, NRE; 43.86 ± 7.58 ppm, WRE; 43.54 ± 6.88 ppm, WRSE) and zinc (40.23 ± 3.82 ppm, NRE; 36.92 ± 4.35 ppm, WRE; 36.7 ± 3.12 ppm, WRSE) was many folds higher than those of uncooked non-fortified rice. This level of iron was found effective in control of iron deficiency anemia (Sarkar et al., 2016; Azam et al., 2017b).

5. Conclusion

Present study showed that washing of fortified and non-fortified rice before cooking significantly reduces iron and zinc content. In comparison to the non-fortified rice, higher loss of iron and zinc occurs in fortified rice. In spite of higher loss in fortified rice, the retained iron and zinc content is many folds higher than that of non-fortified rice. Among the cooking methods the minimum significant loss occurred when rice samples were washed and cooked in rice cooker. Fortified rice prepared by parboiling process and tested in this study can be used to deliver iron and zinc to population groups that might be using a wide range of cooking methods. Therefore, recommending an appropriate cooking method helps in retaining micronutrient content in cooked rice, thereby increasing the intake of iron and zinc especially in the vulnerable groups to alleviate malnutrition and improve health.

CRediT authorship contribution statement

M. Mohibbe Azam: Conceptualization, supervision, validation and Writing - original draft. **Sarla Padmavathi:** Investigation, Formal analysis. **R. Abdul Fiyaz:** Formal analysis, Validation. **Amtul Waris:** Visualization, Methodology. **K.T. Ramya:** Software, Writing - review & editing. **Chirravuri N. Neeraja:** Methodology

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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