Changes in physical, cooking, textural properties and crystallinity upon iron fortification of red rice (*Jyothi*)

Shruti Pandey¹ · Asha MR² · Jayadep A¹

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Abstract The present study is an investigation, into one of the varieties of paddy (*Jyothi*) its fortification with sodium iron EDTA salt, its physical properties, cooking characteristics, color, texture and characterization studies. Fortification was done at two concentrations 1.88 g/kg (T1 corresponding Iron content 0.25 g/Kg) and 3.39 g/kg (T2 corresponding Iron content 0.45 g/Kg). It was found that the iron content increased 2.5 folds after fortification, as compared to control sample. The results revealed that, there was significant difference between the physical properties of rice in the control, fortified brown and milled rice. The cooking parameters for control and fortified brown rice viz. cooking time, volume expansion water uptake were quite close to the control sample. The cooking time for fortified rice was 1–2 min high as compared to control, other parameters remained same. In case of milled rice, the cooking time and volume expansion for control milled rice and fortified was almost same; however the water uptake was higher for fortified milled rice as compared to control milled rice. The texture of fortified brown rice was hard as compared to fortified milled rice, although the taste was found satisfactory. The colour was significantly different in brown and milled rice. X-ray diffraction results revealed the

polycrystalline nature of the fortified sample, was somewhat less crystalline as compared to control sample.

Keywords Fortification · Iron deficiency anaemia · Sensory features · Physical properties · Colour

Introduction

Rice (*Oryza sativa* L.) is a basic food for a large part of the world's population (Juliano 1985). About 90 % of the world's rice is grown and consumed in Asia (Tyagi et al. 2004). There are red and black paddy varieties which have about 38 % more protein, about 18 % more crude fiber and are richer in lysine, vitamin B₁ and other minerals compared to conventional varieties (Chaudhary and Tran 2001). In addition to good quality protein, high fiber content, and vitamin content, pigmented rice varieties have the potential to promote human health because they contain antioxidative compounds that have the ability to inhibit the formation or to reduce the concentrations of reactive cell-damaging free radicals (Acquaviva et al. 2003). Anthocyanins have been recognized as health-promoting functional food ingredients, located in outer layers of the rice kernel, which possess antioxidant activity, anticancerous, hypoglycemic and have anti-inflammatory effects. Colored rice (black and red) consumption is rapidly growing due to their healthy functional food ingredients. A lot of work pertaining to polyphenolic and fiber content has been done in pigmented paddy; however fortification of this paddy with iron has not been attempted. Iron fortification is the optimal approach to reduce the high prevalence of Iron deficiency anaemia (Cook and Reusser 1983). Fortification programmes can be designed to reach either the whole population or be targeted at the groups most at risk for iron deficiency. The success of iron fortification depends on both the

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✉ Shruti Pandey
shruti@cftri.res.in

¹ Department of Grain Science and Technology, CSIR-Central Food Technological Research Institute, Mysore, Karnataka 570020, India

² Human Resource Development, CSIR-Central Food Technological Research Institute, Mysore 570020, India

fortificant and the food vehicle. The bioavailable forms of iron are chemically reactive and often produce undesirable effects when added to food. Iron compounds that have a high bio-availability, and whose absorption is not so susceptible to the negative effects of inhibitory ligands, would be ideal way of administering iron via food (Davidsson et al. 1994).

So far only one compound which fulfills these criteria has been proposed, is the iron chelate Sodium iron (III) EDTA. (Ethylene diamine tetra acetic acid $C_{10}H_{16}N_2O_8$). It has been reported by Martines et al. 1979, that Iron from this compound is better absorbed from the foods containing many inhibitors of iron absorption and has less organoleptic problems as compared to other iron salts. With all this background and insights, in the present study attempt has been made to fortify paddy with iron using sodium iron EDTA salt through hydrothermal treatment. Further the effect of fortification on the physical properties, cooking quality and characterization studies were carried out.

Methods and materials

Material

The paddy (*Jyothi*) was procured from, Agriculture Products Marketing Co-operative (APMC), Bandipalya, Mysore, Karnataka, India, which was harvested in July, 2013. Samples were cleaned, placed in storage bins, stored at 4 °C.

Methods

Parboiling and fortification of paddy

The paddy (1 kg) was soaked in previously boiled water. Iron EDTA ($C_{10}H_{12}FeN_2NaO_8 \cdot 3H_2O$), was used for fortification, as it is highly stable, constant and unlike $FeSO_4$, it does not promote rancidity during storage of iron fortified cereals. It is an approved iron fortificant in grain products. It remains stable during processing and storage and causes fewer organoleptic changes. Iron EDTA complex administered as iron fortificant exchanges completely with vegetarian food iron in the lumen of the gut and has the advantage that the absorption from both extrinsic and intrinsic food iron is higher than that observed when other iron salts are used. The comparison between the iron absorption, from $FeSO_4$ and Iron EDTA as iron fortificant, indicates that the absorption from EDTA is about twice as high than that observed from ferrous sulphate. Absorption ranges from 8 to 13 %. It is also stable during processing. At low pH EDTA acts as a chelating agent and as such prevents iron from binding to phytic acid or phenolic compound, which would otherwise inhibit iron absorption. Its addition enhances the absorption of both food iron and soluble iron fortificant.

The iron salt added was according to two concentrations 1.88 g/kg (T_1 corresponding Iron content 0.25 g/Kg) and 3.39 g/kg (T_2 corresponding Iron content 0.45 g/Kg), in the hot water and the mix was stirred, floating chaff removed and the vessel covered with jute sacks and left overnight to saturation level (30 to 32 % moisture, w.b), (Sashikala et al. 2005). The recommended dietary allowance for iron is 30 mg/day for adult women. The rationale behind adding these two concentrations was to have atleast one third of the iron incorporated through fortification. Two different salt concentrations (0.25 g/kg and 0.45 g/kg) were taken to understand the effect of different concentration on the fortification process; hence T_1 and T_2 were taken for the study. The autoclaving temperature was 100 °C. However, no pressure was used, for parboiling. It was carried out at atmospheric pressure.

Shelling/dehusking

A weighed sample (100 g) of paddy (Control, T_1 & T_2) was dehusked using the Laboratory Satake Sheller (Satake Corporation Tokyo, Japan). The shelled rice and husk were weighed separately for determining the yield of brown rice/dehusked rice, husk, chaff and broken in percentage. The broken ($\frac{3}{4}$) were determined using rectangular perforated trays, with holes (diameter of hole-5 mm) engraved in it.

Milling/polishing

The shelled rice (Control, T_1 & T_2) approximately 150 g, was subjected to milling/polishing using a Mc-Gill miller for 1 min with a pressure of 1 lb. The bran was removed from the dehusked rice by sieving through a 18 mesh sieve and degree of Polish was calculated on weight basis. The broken ($\frac{3}{4}$ & $\frac{1}{2}$) were determined using rectangular perforated trays, with holes (diameter of hole-4 & 5 mm) engraved in it.

Iron estimation

The iron content was estimated as per outlined in AOAC 2000. The samples obtained were as follows, PJ-BR (control brown rice), PFJ-BR- T_1 (Fortified brown rice T_1), PFJ-BR- T_2 (Fortified brown rice T_2), PJ-MR (Control milled rice), PFJ-MR- T_1 (Fortified milled rice T_1) & PFJ-MR- T_2 (Fortified milled rice T_2).

Physical properties

The dimensional parameters viz., grain length (L) breadth (B) and thickness (T) was measured with vernier calipers and L/B, B/T ratio and Normalized grain weight were calculated. The Gravimetric properties viz., weight of 1000 kernels of each sample was determined. The bulk density was determined

using the mass/volume relationship where as the true density was determined using the kerosene displacement method. Porosity was calculated with the help of values derived from Bulk and True density. The frictional properties viz., angle of repose and angle of friction (rough and smooth surface) of the parboiled and fortified brown and milled rice were determined according to the method of Bhattacharya et al. 1972.

Colour

The L^* , a^* and b^* colour values of the parboiled and fortified, cooked brown and milled rice of *Jyothi* were measured using Colour Measurement Spectrophotometer (M/S Hunter lab scan XE model) with a view angle of 2° . In the Hunter system, positive 'a' value indicates redness and negative value indicates the greenness, the neutral is 0. Positive 'b' value indicates yellowness and negative 'b' indicates blue and 0 is neutral. 'L' value represents lightness or brightness, where 0 is black and 100 is white.

Cooking characteristics

Cooking time of parboiled rice was estimated as per the method of Guha and Ali 1998. Water uptake & Volume expansion were carried out by using the method Bhattacharya et al. 1972.

Textural properties

The texture measurement of parboiled and fortified brown and milled cooked rice was done with Lloyd (LR5K) texture meter, by applying load of 1 KN and speed 50 mm/min.

Sensory profiling

Sensory evaluation was conducted under white fluorescent light, with the booth area maintained at temperature $22 \pm 2^\circ\text{C}$ and RH $50 \pm 5\%$. A suitable score card was developed using "Free-Choice Profiling" method selecting suitable terminology. Quantitative Descriptive Analysis (QDA) was used to assess the quality of samples. These are presented as Sensory profiles.

Fourier transform infrared spectroscopy (FTIR)

FT-IR spectra of parboiled and fortified brown and milled rice were measured with FT-Raman module (Thermo Electro Corporation, Japan) using OMNIC software. All pellets were prepared by mixing KBr and sample in w/w proportion. The blended mixture was used for each pellet, which was then put into the sample holder of the FT-IR instrument. The spectra were obtained at a resolution of 4 cm^{-1} in the range 4000 to 400 cm^{-1} .

Scanning electron microscope (SEM)

Scanned images of the fortified rice flour were obtained with a scanning electron Microscope (LEO 435VP, LEO Electron Microscopy Ltd., Cambridge, Electron). The flour was mounted on round aluminum stubs with the aid of double-sided adhesive tape. The samples were coated with gold ($\sim 100\text{ }\mu\text{m}$) by means of a KSE 24 M high vacuum evaporator and scanned. The selected regions were photographed at $200\times$, scale $100\text{ }\mu\text{m}$.

X-ray diffraction (XRD)

The micro-structure of powder samples of parboiled and fortified brown and milled rice were investigated by state-of-the-art technique like X-ray Diffractometer (XRD, Bruker D₂ Phaser system) with Cu K α radiation operating at 30 kV and 10 mA having wavelength (λ) of 0.154 nm. All the measurements performed under θ - 2θ geometry. Therefore, one cannot smear it out the substrate signals at higher 2θ angles between 10 and 45° . Considering this, the substrate scan or background scan (due to the scattering from sample holder and air) has been performed simultaneously under similar conditions. Before calculating d-spacing as well as size of crystallites and the percentage of crystallinity, all the background scans from their corresponding paddy scans were subtracted.

Statistical analysis

The entire experiments were performed in triplicates, and data are presented as mean $\pm$ standard deviation (SD). All statistical analysis was done using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA). Data were assessed for significant difference employing one way ANOVA and were considered to be statistically significant at $p < 0.05$.

Results & discussions

Parboiling & fortification

Fortification In the present study an increase in iron content of about 1.75–2.5 folds was observed for the milled and brown rice. One of the significant advantages of the parboiled rice is that parboiling resulted in a significant inward movement of the fortified Fe into the endosperm, countering milling-induced Fe loss in raw rice grains due to the restricted distribution of Fe in the aleurone and embryo (the bran fraction) of Brown rice (Thai et al. 2011). It was reported that most of the fortified Fe in the milled rice grains remained in the dilute acid-soluble pool, which is considered a potentially bioavailable in the human diet in all cultivars (Swain et al. 2003).

Shelling/dehusking

The results revealed that (Table. 1A), the husk content was almost same for all the samples, however chaff was less for the control sample as compared to fortified. The brokeners were found to be less in control, followed by T2 and T1. The head rice yield was highest for control, followed by Fortified T2 & T1. Hence, it can be concluded that fortification did not affected the yield of the head rice. It has been reported that, increase in head rice yield after parboiling process is observed because of increased tensile strength of kernel by starch granules inside the rice kernel after being gelatinized, thus resisting milling operation and reducing the grain breakage (Pamsakhorn and Noomhorm 2008).

Polishing

In case of polishing it was observed, that the Head rice (Table 1B), was highest for control (84 %) followed by T1 (78 %) and then T2 (77 %). The degree of Polish was almost same in all the three polished samples; however the quantity of three fourth ($\frac{3}{4}$) rice and half ($\frac{1}{2}$) were highest in T1, control, followed by T2.

Iron estimation

The reported increase in iron content in milled rice was 0.0321 g/Kg and 0.0453 g/Kg (the calculations shown here are purely based on the content of iron absorbed by the fortified rice, not on the iron content leached out in water). The actual increase in iron content has been taken into account after comparing from the control sample. Whereas, the value of Iron content in milled control rice was 0.0186 g/Kg, which increased to 0.0321 g/Kg, in case of T₁ and in case of T₂ it increased to 0.0453 g/Kg. The increased values are correspondingly 1.75 fold (T₁) and 2.45 fold (T₂) iron content of control rice sample.

Table 1 (A, B) Shelling and milling of control and fortified paddy/rice

Paddy	PJ-BR	PFJ-BR-T1	PFJ-BR-T2
(A) Dehulling of Paddy			
Husk	18.96 ± 0.12	18.86 ± 0.12	18.98 ± 0.12
Chaff	0.89 ± 0.14	0.98 ± 0.13	0.99 ± 0.14
Head rice	78 ± 0.15	75 ± 0.09	77 ± 0.15
Brokeners	2.15 ± 0.09	5 ± 0.07	3.03 ± 0.09
(B) Milling of Brown rice			
Milled rice	84.6 ± 0.14	78 ± 0.13	77.33 ± 0.13
Three fourth	12 ± 0.13	13 ± 0.08	10.66 ± 0.10
Half	6 ± 0.14	7 ± 0.09	5.92 ± 0.12
Degree of Polish	4.33 ± 0.13	4.66 ± 0.11	4.66 ± 0.14

Physical properties

Physical properties of rice are of paramount importance in all the activities of production, preservation & utilization of rice. Knowledge of physical properties of rice is necessary right from harvesting, drying, handling & storage to milling, packing, cooking, product development and utilization. The length, breadth and thickness of the brown rice grains was almost same after fortification. There was no significant difference (Table 2) in the length, breadth and thickness of milled and brown rice. The normalized grain weight varied significantly between the milled and the brown rice. Coming on to the gravimetric properties, it was found, that the fortified grains had a higher kernel wt and true density as compared to control, in case of brown rice. Same pattern was also observed in the case of milled rice. However the bulk density remained more or less the same for both the brown and milled fortified rice. Bulk density is affected by grain shape, so slender the shape, higher the porosity and lower the bulk density, and hence we can observe that the bulk density is less for milled grains as compared to brown grains. Coming on to the frictional properties it was observed that the, angle of repose was higher for fortified brown rice as well as the milled rice, as compared to control. The static coefficient of friction of rice kernels was determined against the surfaces glass & sandpaper measuring 40- to 60-grit with slope controller mechanism. Angle of friction was higher on the rough surface as compared to smooth surface. All the frictional properties were higher for milled rice as compared to brown rice. This fact was expected because the milling operation makes the grain surface smoother and hence, the friction and consequently its coefficient are affected mainly by the nature and type of the surface in contact (Mohsenin 1986).

Color

Parboiling affects the color of milled rice. Color of parboiled brown rice changes from white to yellow upon altering different processing parameters including the soaking water temperature, duration of soaking and heating duration (Bhattacharya and Subba Rao 1966). There was no significant change in lightness, of the brown rice after fortification. Coming on to the, milled rice we observe there is an increase in the brightness in the milled samples. Overall we can observe that, there was a significant difference in the colour profile of the milled and the brown rice with respect to lightness, redness, yellowness and darkness. Coming on to 'a' & 'b' value we can observe a significant decrease in the fortified brown rice as well as milled rice samples. It has been reported that changes in b value of parboiled brown rice were mainly caused by maillard type non enzymatic browning (Pillaiyar and Mohandas 1981). From the Table 3, we can see that the b value decreased as the level of fortification increased. Hence

Table 2 Physical properties of fortified brown and milled rice

	PJ-BR	PFJ-BR-T1	PFJ-BR-T2	PJ-MR	PFJ-MR-T1	PFJ-MR-T2
Dimensional properties						
Length (mm)	6.7 ± 0.09	6.8 ± 0.09	6.7 ± 0.09	6.5 ± 0.05	6.5 ± 0.02	6.5 ± 0.05
Breadth (mm)	2.2 ± 0.03	2.1 ± 0.03	2.2 ± 0.03	2.1 ± 0.06	2.1 ± 0.02	2.1 ± 0.05
Thickness (mm)	2.2 ± 0.05	2.2 ± 0.05	2.2 ± 0.05	2.1 ± 0.05	2.1 ± 0.09	2.1 ± 0.03
L/B	2.9 ± 0.03	3.2 ± 0.03	3.0 ± 0.03	3.09 ± 0.05	3.09 ± 0.05	3.09 ± 0.07
B/T	1.0 ± 0.05	1.0 ± 0.05	0.9 ± 0.05	1.16 ± 0.02	1.16 ± 0.06	1.16 ± 0.09
Normalised kernel weight (mg/mm)	36.8 ^a ± 0.24	37.7 ^a ± 0.24	39.8 ± 0.24	34.40 ^b ± 0.16	33.6 ^{b,c} ± 0.24	34.46 ^{b,c} ± 0.12
Gravimetric Properties						
1000 kernal Weight (g)	22.4 ^a ± 0.07	22.9 ^{a,b} ±	23.7 ^{c,d} ± 0.07	21.1 ^e ± 0.02	21.3 ^{a,e'} ± 0.05	22.9 ^{a,b,d} ± 0.05
True Density(g/ml)	1.35 ^a ± 0.08	1.44 ^{a,b} ± 0.08	1.47 ^{a,b,c} ± 0.08	1.42 ^{a,b,d} ± 0.10	1.51 ^{b,c,d,e} ± 0.02	1.51 ^{b,d,e} ± 0.03
Bulk density (g/ml)	0.860 ± 0.03	0.860 ± 0.03	0.860 ± 0.03	0.832 ± 0.05	0.832 ± 0.12	0.833 ± 0.17
Porosity (%)	36.29 ± 0.00	40.22 ^a ± 0.00	44.20 ^b ± 0.00	41.40 ^a ± 0.00	44.90 ^{b,c} ± 0.05	44.86 ^{b,c} ± 0.02
Frictional Properties						
Angle of Repose(°)	36 ± 0.03	40 ^a ± 0.03	40 ^{a,b} ± 0.04	41 ^{a,b} ± 0.05	45 ^c ± 0.04	45 ^c ± 0.02
Angle of Friction (°) on Rough surface	30.0 ^a ± 0.37	30.00 ^{a,b} ± 0	29 ^{a,b} ± 0.37	38.91 ^c ± 0.3	38.12 ^{c,d} ± 0.	38.93 ^{c,d} ± 0.25
Angle of Friction (°) on Smooth surface	23.3 ± 0.35	22.05 ^a ± 0	22.45 ^a ± 0.35	26.25 ± 0.26	31.63 ^b ± 0	31.93 ^b ± 0.26

Means and Standard deviation of triplicate values are given. Values within the rows with same super script are not significantly different ($P \leq 0.05$)

we can conclude that some level of coloration was imparted by the salt to the paddy. Significant difference was also observed within the fortified brown rice and milled rice when compared to their control samples.

Cooking characteristics

Cooking quality is important characteristic of rice. When rice is cooked it absorbs water, swells, volume expands and starch granules swell. Grain expands in all the dimensions but

usually more in length. The hardness of grains is greatly affected by the conditions of parboiling like moisture content, the extent of gelatinization and retrogradation (Islam et al. 2004). Cooking time (Table 4) depended on several factors, not only parboiling process, but rice variety and storage time (Hogan 1963). Another quality that relates to cooking time is water absorption, which in turn influences volume expansion of cooked rice. *Indica* has high amount of amylose starch & hence high water uptake is seen. The fine grained varieties have high surface area/unit weight and so high water uptake.

Table 3 Color and textural properties of fortified brown and milled rice

	PJ-BR	PFJ-BR-T1	PFJ-BR-T2	PJ-MR	PFJ-MR-T1	PFJ-MR-T2
Color of Fortified Brown and Milled rice						
L*	33.18 ^a ± 0.04	33.09 ^a ± 0.04	32.99 ^a ± 0.03	48.60 ± 0.07	47.86 ^c ± 0.05	47.66 ^c ± 0.04
a*	11.11 ± 0.04	10.65 ± 0.05	9.99 ± 0.02	9.62 ± 0.05	8.92 ^a ± 0.07	8.83 ^a ± 0.04
b*	8.44 ^a ± 0.02	8.17 ^a ± 0.02	7.17 ± 0.02	9.50 ^b ± 0.09	9.34 ^{b,c} ± 0.05	9.20 ^{a,b,c} ± 0.06
E	65.5 ^a ± 0.06	66.01 ^{a,b} ± 0.06	66.08 ^{a,b} ± 0.07	50.91 ^c ± 0.06	51.11 ^{c,d} ± 0.07	51.50 ^{c,d} ± 0.08
Textural properties of Fortified Brown and Milled rice						
Hardness	1.66 ^a ± 0.15	1.87 ^b ± 0.14	1.91 ^{b,c} ± 0.25	1.54 ^{a,d} ± 0.12	1.68 ^{a,d,e} ± 0.23	1.74 ^{a,b,c,e} ± 0.21
Cohesiveness	0.37 ± 0.17	0.31 ^a ± 0.14	0.29 ^a ± 0.17	0.08 ^b ± 0.14	0.06 ^{b,c} ± 0.20	0.04 ^{b,c} ± 0.14
Springiness	0.60 ^a ± 0.15	0.85 ^{a,b} ± 0.23	0.90 ^{a,b,c} ± 0.30	0.53 ^{a,b,c,d} ± 0.20	0.50 ^{a,b,c,d,e} ± 0.24	0.62 ^{a,b,c,d,e} ± 0.36
Gumminess	0.27 ^a ± 0.17	0.36 ^{a,b,c} ± 0.21	0.38 ^{a,b,c,d} ± 0.14	0.11 ^{a,c} ± 0.06	0.20 ^{a,c,d,e,f} ± 0.16	0.21 ^{a,e,f} ± 0.36
Chewiness	0.32 ± 0.21	0.35 ± 0.05	0.40 ± 0.09	0.07 ± 0.08	0.14 ± 0.01	0.20 ± 0.12
Fracture force	1.32 ± 0.04	1.45 ± 0.12	1.65 ± 0.16	0.18 ^a ± 0.08	0.21 ^{a,b} ± 0.14	0.23 ^{a,b} ± 0.12
Adhesiveness	0.02 ± 0.13	0.04 ± 0.14	0.06 ± 0.16	0.03 ± 0.16	0.03 ± 0.15	0.05 ± 0.20
Stiffness	3.20 ^a ± 0.22	4.41 ^b ± 0.22	4.45 ^b ± 0.21	3.13 ^{b,c} ± 0.25	3.21 ^{a,c,d} ± 0.29	3.39 ^{a,c,d} ± 0.19

Means and Standard deviation of triplicate values are given. Values within the rows with same super script are not significantly different ($P \leq 0.05$)

Table 4 Cooking characteristics of fortified brown and milled rice

Cooking Properties	PJ-BR	PFJ-BR-T1	PFJ-BR-T2	PJ-MR	PFJ-MR-T1	PFJ-MR-T2
Volume Expansion (Ratio)	1.12 ± 0.06	1.13 ± 0.08	1.11 ± 0.10	1.28 ± 0.08	1.26 ± 0.09	1.38 ± 0.10
Cooking time(min)	42 ± 0.06	45 ± 0.13	47 ± 0.19	32 ± 0.12	32 ± 0.13	32 ± 0.03
Water uptake g/g)	2.30 ± 0.13	2.11 ± 0.06	2.29 ± 0.09	3.61 ± 0.08	3.71 ± 0.10	3.91 ± 0.12

In case of parboiled rice, the starch gets completely gelatinized, apparently sufficient solubilization takes place in order to “cement” these cracks together, thus strengthening the kernel (Priestley 1976). This leads to lesser breakage in the process of dehusking and milling of parboiled paddy. It can be seen in Table 4, that cooking time was less for milled rice as compared to brown rice. It was reported that when more fiber and fat is there in rice, due to bran, cooking time increases and water uptake decreases (Juliano et al. 1964). Hence we can observe here as the brown rice contains bran, the cooking time was more as compared to milled rice. The water absorption and swelling characteristics are dependent not only on the chemical composition of the rice grain but also on the surface area of the kernel (Halick and Kelly 1956). The cell walls of rice endosperm are also important consideration. The point of disintegration of rice grains during cooking is characterized by thin cell walls and protein matrix (Little and Dawson 1960). In general swelling number and water uptake of high amylose rice varieties were found to be more compared to those of lower amylose japonica varieties (Chakrabarthy et al. 1972). The volume expansion (Table 4) was higher for the milled rice as compared to brown rice, the highest volume expansion was observed in PFJ-MR-T2 and the lowest for PJ-BR. The highest water uptake (Table 4) was reported for PFJ-MR-T2 and the lowest for PFJ-BR-T1.

Textural properties

The textural properties play an important role in the consumption of rice. The rice varieties with higher amylose content had superior cooking qualities, giving cooked grains which were flaky as well as dry and on cooling gave relatively firm texture compared to low amylose rice which gave moist, sticky cooked grains which on cooling remained mushy (Chakrabarthy et al. 1972). From Table 3, we can observe that, there was no significant difference between the hardness of fortified cooked brown rice samples, however significant difference was observed for hardness between milled and brown rice. Hardness is the force required to compress food between the molar teeth on the first chew. In technical terms it can be defined as the force exhibited in first bite at maximum compression. Degree may range from soft (low), firm (middle) and hard (high). Overall hardness was high for brown rice as compared to milled rice. The cooked parboiled rice is harder than cooked raw rice. Coming on to cohesiveness we observed,

that the brown rice was more cohesive as compared to milled rice. Cohesiveness is the internal force holding a grain together before it breaks when compressed between the teeth. Degree varies from mushy (low) tender (middle) to leathery (high). No significant difference was observed between the cohesiveness for PFJ-MR-T1 and PFJ-MR-T2. Springiness was higher for brown rice as compared to milled rice. It can be defined as degree to which cooked food returns to its original shape once it has been compressed between the teeth. Degree varies from viscous flow (low) to rubbery (high). There was no significant difference within all the six treatments. Significant difference was also not observed for springiness between PFJ-BR-T1 & PFJ-BR-T2. Gumminess another parameter which is the. Denseness that persist throughout mastication of food. It is the energy required to disintegrate cooked food to a state, ready for swallowing. Degree varies from mealy (low) to gummy (high). No significant difference was observed in gumminess within all the six treatments. Chewiness is the, number of chews required to masticate the food at a constant rate of force application to reduce it to a consistency suitable for swallowing. Significant difference was observed for chewiness within all the six treatments. The fracture force, is nothing but the force required with which the cooked food crumbles. A high degree would be a rice with a high degree of hardness and low degree of cohesiveness. This correlated well with the fact that as the, hardness increased for fortified brown and milled rice, the cohesiveness decreased, and correspondingly fracture force increased. The fracture force was higher for brown rice as compared to milled rice, however no significant difference was observed for PJ-MR, PFJ-MR-T1 & PFJ-MR-T2. Another parameter studied was adhesiveness, which is stated, as the force required removing the food particles that adhere to the mouth during eating. Significant difference was observed for adhesiveness within all the six treatments. Stiffness, in other words, the rigidity of a food item, the extent to which it resists deformation in response to an applied force. Stiffness was high for brown rice as compared to milled rice. Significant difference was observed between control brown and fortified brown rice.

Sensory properties

Samples coded D (control), E (Treatment 1) and F (Treatment 2) were brown in color with varying shades of darkness. The

samples D, E and F were found to have brown color which lacked visual appeal and apparently resulted in decreased overall quality (7.4, 7.2 and 7.1 respectively) indicating lower acceptability. All the samples had perceptible bran-like aroma, comparable cooked rice aroma and moderate sweet taste. There was no perceptible off odor or off taste in the samples.

Fourier transformed infrared spectroscopy (FTIR)

The spectrum for fortified brown and milled rice are given in Supplementary Figure 1. Comparing the above three spectrums, (Fig. 1a, b, c) one can note the following major changes involving the hydroxyl & carboxylic groups of the fortified and control brown rice. The characteristic $\nu_{as}(\text{NH}_2)$ and $\nu_s(\text{NH}_2)$ bonds of the spectrum at (Fig. 1a) 3316 cm^{-1} are non symmetrically shifted to 3309 cm^{-1} in Fig. 1b, whereas 3308 cm^{-1} in Fig. 1c. The broad intensive (OH) envelope related to associated carboxylic group centered at 3004.0 cm^{-1} (Fig. 1a) shifts to 3008 cm^{-1} (Fig. 1b) and 3016 cm^{-1} (Fig. 1c) cm^{-1} . Presence of $\text{C}\equiv\text{C}$ alkyne group is observed at 2152 cm^{-1} (Fig. 1b) and 2140 cm^{-1} (Fig. 1c) respectively. A new peak was observed in Fig. 1b at 3744 cm^{-1} .

In Fig. 1d, e & f, we can see the spectrum for control & fortified milled rice. The peak in Fig. 1d centered at 3744 cm^{-1} shifts to 3732 cm^{-1} (Fig. 1e) and 3740 cm^{-1} (Fig. 1f). Two new peaks are seen in Fig. 1e & f at 3008 cm^{-1} & 3012 cm^{-1} respectively. A single new peak is also observed at Fig. 1f, centered at 3164 cm^{-1} .

Scanning electron microscope (SEM)

Morphological characteristics like shape, size and distribution of starch granules are attributed to the biological origin of the grain (Vandeputte and Delcour 2004). In the case of brown rice, the individual granules were irregular (Fig. 1a, b & c); with noticeable flat areas due to compact packing in the grain endosperm. Some spherical starch granules were also present. The individual granules, in the case of rice starch, develop into compact spherical bundles or clusters, known as compound granules, which fill most of the central space within the endosperm cells (Singh et al. 2003). Physico-chemical properties, such as percent transmittance, amylose content, swelling power and water binding capacity were significantly correlated with the average granule size of the starches separated from different plant sources (Singh and Singh 2001). As regards to the size, most frequent were granules with diameter $2\text{--}5\text{ }\mu\text{m}$. In the case of milled rice Fig. 1 d, e & f few agglomerated structures were observed. Few sporadic breakings were observed in these images which might be due to starch swelling due to hydrothermal treatment. As regards to the size, most frequent were granules with diameter $4\text{--}6\text{ }\mu\text{m}$. Under the effect of water and high temperature, starch granules underwent gelatinization, as reported by other researchers.

X-ray diffraction (XRD)

The X-rays are indispensable tools for identifying some special features of Fe based salts, their composition & structure as

Fig. 1 SEM of fortified brown and milled rice at $200\times$, scale $100\text{ }\mu\text{m}$. **a** PJ-BR. **b** PFJ-BR-T1. **c** PFJ-BR-T2. **d** PJ-MR. **e** PFJ-MR-T1. **f** PFJ-MR-T2

well as describing particle size, surface morphology, micro structure, crystal structure and other physical properties. It also helps in measuring the average spacing between layers or rows of atoms, determine the orientation of a single crystal or grain & finding the crystal structure of an unknown material. Under the scan performed in the present study, the sample is scanned along the surface normal direction & by doing this detector is cutting all the planes which are lying parallel to the surface. We can derive information about the vertical structure of the sample. The sample was fixed to the X-ray source. θ - 2θ scans were performed in which both the arms move with the same angle. The X ray diffraction scan of fortified (T1 & T2) and control rice flour is illustrated in Fig. 2 (brown rice in Fig. 2.1 and milled rice Fig. 2.2). From Fig. 2.1 we can infer, that all the 3 samples have polycrystalline nature due to appearance of number of peaks. Every peak corresponds to one set of plane lying randomly in the powdered sample. It can also be observed that, all the three samples are sum of amorphous and crystalline parts. In Fig. 2.1, four stronger diffraction peaks were observed at 2θ values of 15.41° , 17.90° ,

20.18° and 23.07° . At the same time we can observe that all the dominant peaks are sitting at the amorphous halo parts centered at 18.8° and 25.2° . We can also observe here, that, the peak starting intensity is different for PJ-BR; this could be due to difference in amorphous content or due to higher background which is dominating at lower 2θ value. As the scanning time is same for each individual points in all the 3 scans, one can see less intensity and less dominating amorphous part in the control sample (PJ-BR). This indicates that the presence of number of crystallites corresponding to each individual set of planes is less for the controlled sample. At the same time all the samples contain different amorphous fractions and background due to this one has to subtract the background before computing the individual peaks. After subtracting background due to air and glass scattering & using peak fitting (under origin software) one can get peak centre, peak area & Full Width Half Maximum (FWHM). The inter planar distance “d” has been derived from *Bragg Law* $n\lambda = 2d \sin\theta$. Interplanar distance can be calculated from the formula $d = n\lambda/2\sin\theta$, where θ , is the angle of incidence, and d is

Fig. 2 XRD images of fortified brown and milled rice. XRD image of brown & control fortified rice. XRD image of milled & control fortified rice

the Interplanar distance. The size of crystals (γ) can be calculated using Sherrer's formula ($\gamma = \frac{K*\lambda}{B*\cos\theta}$), where γ = size of crystallite, K = constant (0.9), B = FWHM (full width at half max), θ = angle of Incidence. The X-ray diffraction pattern was comparable to those reported earlier for rice starches (Singh et al. 2007). The appearance of all the crystalline peaks in the X-ray diffraction pattern of the powdered samples are attributed to the crystalline nature of starch. The granule crystallinity is ascribed to amylopectin components in starch (Deepa et al. 2012). The pattern obtained is comparable with form of starches common in all cereals. Similar results were also reported where in a V-type pattern was observed for cereal grains (Zobel 1988). The higher amount of long chains of amylopectin increases crystallinity (Singh et al. 2006a, b). The difference in crystallinity structures of all the three samples may be attributed to fine structures of each starch component i.e. proportion of amylose fraction present in

individual samples, short and long chains of amylopectin (Noda et al. 1988). In the case of fortified brown rice (Table 5A–C), it can be seen that, there is gradual shift in the peak position. The peak shifts from 15.41° (Table 5A) to 15.34° (Table 5B) and then 15.28° (Table 5C), shifting towards lower θ value. The d spacing is observed to be more or less the same. Coming on to the milled rice, here also there is a shift in the peak position from 15.19 (Table 5D) to 15.18 (Table 5E) and to 15.17 (Table 5F), which is also reflected in the d spacing. Hence we observe that the peak shifts to low θ value, and hence the inter planar distance (d spacing) increases and correspondingly the size of crystals increase. The most probable answer is that in fortified T1 and T2, after adding iron salt, one of the 3 length is also changing in order to accommodate Fe atom. The unit cell structure of rice is trying to reorganize itself, in order to accommodate iron molecule. Decreased crystallinity of the fortified sample indicates

Table 5 XRD analysis of fortified brown and milled rice. PJ-BR (A), PFJ-BR-T1 (B), PFJ-BR-T2 (C), PJ-MR (D), PFJ-MR-T1 (E), PFJ-MR-T2 (F)

Peak	Peak Position (degree)	Peak Area (a.u)	Peak FWHM (degree)	D spacing nm	Size of Crystallites (nm)
PJ-BR (A)					
Peak 1	15.41	148.48	0.737	0.57	11
Peak 2	17.90	314.54	1.366	0.49	6
Peak 3	20.18	127.72	0.829	0.44	10
Peak 4	23.07	156.53	0.992	0.39	8
PFJ-BR-T1 (B)					
Peak 1	15.34	323	0.697	0.58	12
Peak 2	18.18	489	0.903	0.49	9
Peak 3	20.28	180	0.827	0.44	19
Peak 4	23.24	758	1.0113	0.38	8
PFJ-BR-T2 (C)					
Peak 1	15.28	288.00	0.910	0.58	9
Peak 2	17.94	447.19	1.563	0.49	5
Peak 3	20.13	102.35	0.708	0.44	11
Peak 4	23.07	116.68	0.865	0.39	9
PJ-MR (D)					
Peak 1	15.19	216	0.600	0.58	13
Peak 2	17.86	413	1.285	0.50	6
Peak 3	20.05	298	0.994	0.44	8
Peak 4	23.11	469	1.140	0.38	7
PFJ-MR-T1 (E)					
Peak 1	15.18	172	0.557	0.58	14
Peak 2	17.66	551	1.483	0.50	5
Peak 3	19.94	145	0.847	0.44	10
Peak 4	23.07	585	1.340	0.38	6
PFJ-MR-T2 (F)					
Peak 1	15.17	173	0.587	0.58	14
Peak 2	17.64	497	1.382	0.50	6
Peak 3	19.93	141	0.734	0.44	11
Peak 4	23.04	177	0.803	0.39	10

change in the internal structure and change in one of its lengths (varying d-spacing) could be due to that the unit cell of rice/starch try to reorganize itself, in order to accommodate the Fe atom. This weakens the crystal lattice of rice/starch too. Resultant, the decrease in its crystallinity. It also shows the penetration of iron in the rice grain and further confirms fortification of rice with iron structurally. If the rice remains crystalline then absorption/attachment of iron cannot permanent i.e., It would be a physical absorption not a chemical absorption. Therefore, the reduction in crystallinity also indicates the well absorption of Fe into the starch unit cell. However it can be concluded that both the brown and the milled rice behave differently, in the case of brown rice, the size of crystals increase whereas in case of milled rice the number of crystals increase.

Conclusion

The present study pioneered an investigation, into one of the varieties of paddy (*jyothi*) and its fortification with sodium iron EDTA salt. Fortification was done at two concentrations, 1.88 g/kg (T1 corresponding Iron content 0.25 g/Kg) and 3.39 g/kg (T2 corresponding Iron content 0.45 g/Kg). It was found that the iron content increased 1.75–2.5 folds after fortification in milled fortified rice. The present research focused on the physical properties, cooking characteristics, color, texture and characterization (FTIR, XRD and SEM) studies of the brown and milled fortified rice. The results revealed that, there were significant changes in the physical properties of rice in the control and fortified brown and milled rice samples. The length of the milled rice samples was less as compared to brown rice which is due to the polishing given to brown rice. It was also observed, that the density for milled rice (1.4 g/ml) was higher as compared to brown rice (1.3 g/ml), irrespective of the treatments. Observation's recorded for cooking properties revealed, that the water uptake and volume expansion were found to be higher for parboiled and fortified milled rice as compared to brown rice. The texture of fortified brown rice was hard as compared to fortified milled rice. It was observed that the fracture force for brown rice was higher as compared to milled rice. The colour difference in the fortified and non-fortified rice was significantly, different. Milled rice was found to be brighter than brown rice. The taste of the cooked fortified polished rice was found to be satisfactory. X-ray diffraction measurements were explored to understand the difference in the structural properties, as well as, the crystallinity in the fortified and control rice samples. Results reveal the polycrystalline nature of the fortified sample was somewhat less crystalline as compared to control rice sample. The above technology after up-scaling and carrying out the shelf life studies will be further transferred to industries or NGO's

who are working on food fortification. Bio-accessibility studies will also be taken up.

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Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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