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Effect of iron and folic acid fortification on *in vitro* bioavailability and starch hydrolysis in ready-to-eat parboiled rice

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Abstract:
Iron (Fe) and folic acid (FA) fortified parboiled rice was produced by applying 'brown rice parboiling' method. The effect of milling and the effectiveness of fortification were tested in relation to the amount of bioaccessible and bioavailable form of Fe and FA. An in vitro starch hydrolysis assay was employed to assess the effect on simulated glycaemic index (GI). The % bioaccessibility of Fe and FA in the unmilled fortified rice were in the range of 57.6 to 65.8%, and 55.1 to 91.9%, respectively. The % bioavailability in the unfortified parboiled rice was negligible as compared to Fe (14.7 to 32.1%) and FA (13.5 to 27.5%) fortified rice. The GI of unfortified and fortified parboiled rice samples was in the range of 56-69, which was lower than the raw rice. The results demonstrated that this approach can be a novel and rapid method to produce micronutrient enhanced ready-to-eat rice.

Keywords: Brown rice; Parboiling; Micronutrients; Fortification; Bioaccessibility; Bioavailability; Glycaemic index
Introduction

Micronutrients deficiency is a public health problem and considered to be a matter of concern, especially in the South Asia (Hettiarachchi, Hilmers, Liyanage, & Abrams, 2004). The common causative factors of micronutrients deficiency are inadequate dietary intake, or presence of inhibitors or their absorption in the diet. Deficiency of Fe, iodine, FA, zinc, and vitamin-A is a worldwide concern resulting in deaths of many underprivileged children in the developing countries including India (Kotecha, 2008). Fe deficiency comes under the most important forms of malnutrition (Stoltzfus & Dreyfuss, 1998; Stephenson, 2000), which has affected an estimate of about two billion people worldwide (Lynch, 2005; Hettiarachchi et al., 2004), and it is the sixth highest health risk factor in developing countries (WHO, 2002). On the other hand, FA is involved in the protein metabolism, nervous tissue and nucleic acid synthesis (Choi & Manson, 2002), with homocysteinemia and neural tube defects being the two important diseases in relation to folate deficiency (Krishnaswamy & Nair, 2001). It is estimated that about 0.2 million babies in India are affected every year with neural tube defects, mostly arising from FA deficiency (Kotecha, 2008).

Focus has been on the fortification of micronutrients on staple foods, which can act as good vehicles to eliminate micronutrient deficiency. Efforts are on to enhance micronutrients in rice grains, and fortification is one of the applied techniques to achieve this enhancement. For delivering nutrients to the mass population, coating, dusting, supplementation and bio-fortification methods have not been considered as a cost-effective approach as compared to fortification during parboiling (Prom-u-thai, Fukai, Godwin, Rerkasem, & Huang, 2008). Parboiling is a hydrothermal treatment which consists of soaking, steaming and drying; and
about 15% of the world's milled rice production is consumed in the form of parboiled rice (Bhattacharya, 2004).

There are reports on the fortification and bioaccessibility of Fe in whole grain parboiled rice (Prom-u-thai, Glahn, Cheng, Fukai, Rerkasem, & Huang, 2009), FA in fortified yogurt, and bread (Arkbåge, Verwei, Havenaar, & Witthöft, 2003; Öhrvik, Öhrvik, Tallkvist, & Witthöft, 2010; Chandra-Hioe., 2013). However, there are no reports on the bioaccessibility and bioavailability of Fe and FA fortified parboiled brown rice. In Assam, India, a product named komal chawal, meaning soft rice, is produced from a low amylose variety of paddy called chokuwa simply by parboiling, and is consumed without cooking. Upon soaking for 20-25 min in warm water, this product rehydrates to a texture which is soft enough for consumption (Wahengbam & Hazarika, 2019). In the present study, this rice variety is used as a carrier for micronutrients fortification during the production of komal chawal, with an objective of obtaining ready-to-eat micronutrient rich parboiled rice. We also investigated the bioaccessibility, bioavailability of Fe and FA in the unfortified parboiled rice and fortified parboiled rice, and effect of in-vitro starch hydrolysis in the unfortified and fortified parboiled rice.

2. Materials and methods

2.1 Materials

Chokuwa paddy was obtained from a local farm nearby to the Department of Food Engineering Technology, Tezpur University, Assam. The paddy was dehusked by using a sheller (RTE-07, A-GRAIN, India) to get the brown rice. Caco-2 cells were obtained from the cell culture lab of the Institute of Global Food Security, Queen’s University, Belfast, UK. Media and supplements
for cell line studies were obtained from Gibco and Sigma Aldrich, USA and other chemicals like FeNaEDTA, folic acid and enzymes were obtained from Sigma, USA.

2.2 Production of Fe, and FA fortified parboiled brown rice

Prior to soaking, the chokuwa brown rice (100 g) was washed once with distilled deionised water and the excess water was drained. FeNaEDTA (C_{10}H_{12}N_{2}NaFeO_{8}) and FA (C_{19}H_{19}N_{7}O_{6}) were used as Fe and FA fortificant, respectively, during the soaking steps of parboiling in 1:2 ratio (w/v) of rice to solution at 60°C for 90 min, in replicates of three sets. The concentration ranges used for Fe and FA are given in Table 1. After soaking, the excess solution was drained and the rice was steamed at 1.05 kg/cm^{2} for 10 min in a vertical autoclave, followed by drying at 40°C in lab-scale tunnel tray dryer (Armfield – UOP8-A, UK) till 12% moisture. The products thus obtained were milled separately for 30 s and 60 s in a rice polisher (RTE-08, A-GRAIN, India) and stored at 4°C. Before the in vitro study, the rice samples were rehydrated to cooked condition, and then freeze dried and powdered. The coding for raw (R), unfortified parboiled brown rice (PBR) and Fe and FA fortified rice are given in Table 1.

2.3 In vitro digestibility for assessment of bioassessible Fe and FA

Fe and FA bioaccessibility in the unfortified and fortified parboiled rice was carried out by simulation digestion (Wei et al., 2012; Minekus et al., 2014). In vitro digestion was conducted to simulate the human digestive system via a three-step digestion that includes oral, gastric and intestinal digestion (Wei et al., 2012; Minekus et al., 2014). For the oral step, 5 g of dried rice powder was mixed with 30 ml of 140 mM NaCl and 5 mM KCl solution. Then 0.5 ml of α-amylase (1500U/ml) was added, followed by 25 μl of 0.3 M CaCl_{2} and 500 μl of ascorbic acid
(100 mM) solution and maintained at 37°C for 2 min in a shaking water bath. For the gastric step, 0.5 ml of pepsin solution (11000U/ml) was added to the orally digested sample and maintained at pH 2 (1M HCl) for 2 h in a shaking water bath at 37°C. Then the sample was adjusted to pH 5 (1M NaHCO₃), and the intestinal step was followed with addition of 2.5 ml of pancreatin-bile solution (0.45 g of bile salts and 0.075 g of pancreatin in 37.5 ml of 0.1M NaHCO₃). Then 40 μl of 0.3 M CaCl₂ was added and the pH was adjusted to 7.0 with 1 M NaOH and incubated for another 2 h in a shaking water bath at 37°C. The digested samples were cooled in ice for 10 min and centrifuged at 4600 rpm for 40 min at 4°C. The supernatants were separated, snap frozen in liquid nitrogen and stored at -80°C until further analysis.

2.4 Analysis of Fe and FA

The supernatants (soluble mineral fraction) from the previous step were analyzed for bioaccessible Fe by inductively coupled plasma-optical emission spectrometry (5100 ICP-OES, Agilent Technologies, USA) at 238.20 nm. For bioaccessible FA, supernatants were purified using solid-phase extraction on SAX cartridges (55 μm, 70 Å, 500 mg/6 ml) and further filtered through 0.45 μm cellulose acetate membrane prior to analysis in a HPLC system (Waters 2695, USA) using a photodiode array detector (Waters-2996), monitored at 280 nm. The separation was performed using a Luna 5μ C18(2) column (150 × 4.6 mm, Phenomenex- 00F-452-E0). The solvent flow rate was set at 0.8 ml/min with an increasing rate of solvent B (acetonitrile) over solvent A (30 mM phosphate buffer - pH 2.2), according to Kam, Arcot, and Adesina (2012). Further, the % bioaccessibility was calculated by using equation 1 (Hemalatha, Platel, & Srinivasan, 2007).

\[
% \text{bioaccessibility} = \frac{\text{bioaccessible m.f}}{\text{Total m.c}} \times 100 \quad (1)
\]
Where, bioaccessible m.f is the soluble mineral fraction (Fe/FA) obtained after simulated digestion (mg/100 g rice), and total m.c is the total micronutrient (Fe/FA) content of each rice sample (mg/100 g rice) after the parboiling process before cooking.

2.5 Bioavailability study using Caco-2 cell model

The bioaccessible form of soluble mineral fraction (supernatant) which was obtained after in vitro simulation digestion was used for determining the bioavailable form of Fe and FA transport through Caco-2 cells. Cells were cultured in 75 cm$^2$ flasks and maintained in high glucose (4.5 g/L) minimum essential media (MEM) with 10% (v/v) fetal bovine serum, 1% (v/v) nonessential amino acids, 4 mM L-glutamine, and 1% (v/v) penicillin-streptomycin, and 1% (v/v) sodium pyruvate. The cells were maintained at 37°C in an incubator with 5% CO$_2$, and 95% relative humidity (Gillespie, Pan, Marco-Ramell, Meharg, & Green, 2017).

For testing the bioavailability, 50000 cells/cm$^2$ (2×10$^5$ cells/well) were seeded in polyester membrane inserts whose basal compartment contained complete MEM. The culture medium was changed in every 48 h, till 21 days of initial seeding, after which the growth medium was removed. Then the cell monolayer was washed with Ca$^{2+}$ and Mg$^{2+}$ free Hank’s balance salt solution and the basolateral compartments were filled with transport solution (130 mM NaCl, 10 mM KCl, 1 mM MgSO$_4$, 5 mM glucose, and 50 mM HEPES, pH 7.4) and the apical chambers (inserts) were filled with the soluble mineral fraction followed by incubation at 37°C for 2 h. After then the basolateral compartments were collected for the determination of Fe and FA transport across the cell monolayer. Cell viabilities were assessed by trypan blue exclusion, which were typically 92–97% after 2 h of exposure. The % absorption of bioavailable
Fe and FA transport across the cell monolayer was obtained by using equation 2 (Wei et al., 2012).

\[
\text{% absorption of bioavailable Fe } = \frac{\text{Transport } x_{\text{basolateral}}}{\text{Bioaccessible } x_{\text{apical}}} \times 100
\]  

Where, transport \( x \) equals the amount of Fe/FA in ppm, transported to the basolateral compartment through the cell monolayer and bioaccessible \( x \) in apical compartment are the amount of bioaccessible Fe/FA in ppm.

2.6 In-vitro starch hydrolysis assay

The rate of glucose liberation due to hydrolysis of the solubilized starch was monitored by measuring the activity of \( \alpha \)-glucosidase enzyme on rice. Enzyme stock was prepared by mixing intestinal acetone powders from rat (I1630 Sigma-Aldrich, UK) with citrate buffer in 1:9 (w/v) ratio. Samples were prepared by mixing 300 µl rice starch solution (0.1 g/ml in phosphate buffer saline (PBS) with 150 µl of PBS and 20 µl of \( \alpha \)-glucosidase enzyme was added. The liberation of glucose was measured from 0 to 120 min at regular interval of 30 min by using a Microstat P-GM7 portable analyser (Analox Instruments USA Inc. Lunenburg MA). Glucose was taken as a reference food. The area under curve (AUC) for each hydrolysis curve was obtained and the hydrolysis index (HI) was determined by dividing the AUC of each sample by the corresponding AUC of reference food (Bravo, Siddhuraju, & Saura-Calixto, 1998; Frei, Siddhuraju, & Becker, 2003) and the expected glycaemic index (GI) was calculated according to equation 3 (Bravo et al., 1998).

\[
GI = 39.71 + 0.549 \times HI
\]
2.7 Statistical analysis

Three replications were performed for analytical determinations. One-way analysis of variance was carried out for data analysis and Duncan’s mean comparison test applied at a probability of $P = 0.05$ to determine differences among treatments using IBM SPSS Statistics 20.

3. Results and Discussion

3.1 In vitro digestibility of Fe and FA fortified rice

The bioaccessible form of soluble Fe and FA retained in the supernatant after the in-vitro simulation digestion is the potentially available form for absorption in the intestinal cells and its availability depends on release from the food matrix following digestion. The amount of bioaccessible Fe and FA in the unfortified and fortified parboiled (unmilled and milled) rice are shown in Fig. S1a and S1b, and their % bioaccessibility for Fe and FA are shown in Fig. 1a and 1b, respectively. The % bioaccessibility of R ranged between 5% and 8% for 60 s and 0 s milled samples. Except in samples Fe-1a and Fe-1b, the % bioaccessibility of Fe followed a similar trend of increase with increase in fortification concentration (Fig.1a), which might be due to less variation in between the bioaccessible form and total Fe content. However, in case of FA fortified parboiled rice the % bioaccessibility decreased with increase in concentration from 50 to 200 mg/200 ml water, followed by a slight increase from 400 to 800 mg/200 ml of water. For 0 s milled fortified parboiled rice, the % bioaccessibility ranged from 57.6% to 65.8%, and 55.1 to 91.9%, respectively (Fig. 1a and 1b), which were significantly higher ($p<0.05$) than in PBR. In the 30 s milled Fe and FA fortified samples, the % bioaccessibility ranged from 44.5% to 60.4%, and 56.6% to 96.0%, respectively (Fig 1a and 1b). Similarly, in the 60 s milled Fe and FA fortified samples, the % bioaccessibility ranged from 35.1% to 60.2%, and 62.6% to 91.1%,
respectively (Fig 1a and 1b). In similar study, folate bioaccessibility of 82% in FA fortified yogurt has been reported by Arkbåge et al. (2003), which is within the range obtained in the present study.

In the effect of milling, it was observed that there was a reduction (loss) in bioaccessible Fe and FA content when the milling time was increased from 0 s to 60 s (Fig.S1 a and b). There was a loss of 31.2 to 60.8%, and 4.4 to 25.6% bioaccessible Fe and FA, respectively from 0 and 60 s milling. There was loss in the % of bioaccessible Fe when milling time was increased from 0 s and 60 s, and followed the increasing order of Fe-5a to 5c (31.2%), Fe-2a to 2c (46.0%), Fe-1a to 1c (47.9%), Fe-3a to 3c (54.6%), and Fe-4a to 4c (60.8%). Likewise, the % loss of bioaccessible FA was from FA-5a to 5c (4.3%), FA-3a to 3c (9.4%), FA-4a to 4c (11.7%), FA-1a to 1c (25.5%), and FA-2a to 2c (35.5%). In overall, the % loss of bioaccessible Fe and FA during milling (60 s) was lowest in the highest concentrated fortified rice for both Fe and FA. It shows that at this concentration the migration of fortificant inside the endosperm was more due to availability of more ions in the soaking solution (Fig. S1a and S1b). However, the higher % loss in the Fe-3 and Fe-4 concentrations might be due to variation in the distribution of Fe ions in the rice kernels that led to more reduction while polishing. Such variation might be due to differences in the arrangement of kernel fissures that lead to difference in the migration rate or accumulation in a particular outer surface of bran portion (Prom-u-thai et al., 2008). Although the concentrations of bioaccessible Fe retained in Fe-1 and Fe-2 are less as compared to the rest of the higher concentrations, but they have moderately lesser % loss of bioaccessible Fe. It might be due to very low concentration of soluble Fe ions in the soaking solution, leading to higher accumulation in the bran portion as compared to the endosperm (Prom-u-thai et al., 2008).
On the other hand, the higher % loss of bioaccessible FA in the lower fortificant concentration samples (FA-1 and FA-2) might be due to abridged soluble nature of FA in water. Lesser FA concentration implies a lower concentration of solute in the soaking solution, thereby resulting in a reduced migration to the inner surface of the rice endosperm (Wahengbam, Green, & Hazarika, 2019). However, with increased concentration, the solubility increases and hence the migration. The % loss of bioaccessible Fe in 30 s to 60 s milled samples ranged from 8.6 to 41.9%, with highest loss from Fe-1b to Fe-1c followed by Fe-2b to Fe-2c. Similarly, loss was 11.4 to 25.6% for FA fortified with highest loss in FA-2b to FA-2c. The % loss of both bioaccessible Fe and FA during 0 to 30 s milling was comparatively less than the 30 s to 60 s milling, thereby indicating that the diffusion of Fe and FA might be beyond the aleurone layer. Thus, it can be concluded that, with relatively less concentration in the solution, the diffusion of Fe and FA to the rice grains through aleurone layer during the fortification process (soaking) might be somewhat distributed in the outer endosperm area only, thereby resulting in a higher reduction in Fe and FA content due to milling (Fig. S1a and S1b). Subsequently, in spite of the similar soaking period, the % decrease of bioaccessible Fe and FA in 30 s milled samples was less in higher concentrated Fe and FA fortified samples. This may be due to greater penetration or diffusion of Fe and FA ions to the inner endosperm portion of the rice grains.

3.2 Bioavailable form of Fe and FA in Caco-2 cell model

The bioaccessible Fe and FA present in the supernatant (soluble fraction) filled in the upper apical chamber which was absorbed through Caco-2 cell monolayer into the transport medium is presented as % absorption of bioavailable Fe and FA. The % absorption of bioavailable Fe in PBR was very less and negligible as compared to fortified samples as shown in Fig. 2a. It was
observed that the % absorption of bioavailable Fe and FA in 0 s milled fortified samples were more than the milled counterparts. In 0 s milled samples, with increase in Fe fortification concentration, the % absorption also increased from Fe-1a to Fe-5a. This showed a positive correlation with the increasing level of Fe-fortification. Whereas, in case of FA fortified rice, the % absorption of bioavailable FA slightly decreased from FA-1a to FA-3a. However, in the last two highest fortificant concentrations (FA-4a to FA-5a), the % absorption significantly increased (Fig. 2b). These respective trends were observed in both Fe and FA fortified milled (30 s and 60 s) parboiled rice. It was seen that, increasing the milling time, the amount of bioavailable Fe decreased, however, the values remained well above those in the unfortified parboiled rice. The % absorption of a bioavailable Fe ranged from 11.0 to 27.9% for 30 s milled and 9.9 to 26.2% for 60 s milled fortified rice as shown in Fig. 2a. Similarly, for FA, it ranged from 8.7 to 25.3% for 30 s milled, and 8.1 to 20.2% for 60 s milled rice as shown in Fig. 2b. A significant difference (p<0.05) was observed in the deviation between the % absorption of unmilled and milled FA fortified rice also.

Similar observations have been reported in Fe fortified whole grain parboiled rice by Prom-u-thai et al. (2009). The amount of % absorption of FA in the present study is also comparable with the findings of Chandra-Hioe et al. (2013), who claimed about 14% absorption of bioavailable FA in FA fortified bread (250 μg/100 g flour) and Öhrvik et al. (2010) who found about 6% transport in fortified bread (2.8–12.4 mg/1,000 g final dough). These variations in bioavailability of nutrient might be due the less amount of added fortificant, different digestion methods used, variation in the amount of release from the food matrix, intestinal cells absorption, and transport to body cells, thereby affecting the overall % absorption (Etcheverry, Grusak, & Fleige, 2012).
To observe the effect of concentration of bioaccessible Fe and FA upon absorption in the intestinal cells, a relationship was derived between the amounts of bioaccessible Fe and FA for each sample with their respective % absorption values. A close linear relationship between bioaccessible Fe and the transport of Fe in the form of % absorption was found as shown in Fig. 3a. This relationship was also observed for the FA fortified samples. The goodness of fit ($R^2=0.95$) for linear relationship between concentration of bioaccessible Fe and its % absorption was slightly higher than the respective values ($R^2=0.87$) for bioaccessible FA (Fig. 3b). The results show that increasing the concentration of bioaccessible Fe and FA gave more % absorption.

3.3 In vitro assay simulating GI

The digestible starch of R, PBR, and fortified parboiled rice were hydrolyzed by $\alpha$-glucosidase into glucose and the glucose liberation rate (hydrolysis rate) from 0 min to 120 min were checked for all test groups, and compared with R and PBR. Each group consisted of seven samples as shown in Fig 4a, 4b, and 4c for 0 s, 30 s and 60 s milled samples for Fe fortified rice, respectively; and Fig. 5a, 5b and 5c for 0s, 30 s and 60 s milled samples for FA fortified rice, respectively.

The variation in HI values for R and PBR with Fe and FA fortified rice are shown in Fig. 4d and Fig. 5d, respectively. Moreover, the simulated GIs for R, PBR, and Fe and FA fortified parboiled rice are given in Table-S1. It was observed that the rate of starch hydrolysis increased with increase in enzyme reaction time. For R and PBR, the liberation of glucose response increased with milling time. The glucose liberation rate for R was higher than the PBR and fortified parboiled rice. This may be attributed to the influence of crystalline arrangement in A-
type or B-type starches; as the R rice has A-type starch which gets converted into B or/and V-type or mixed pattern in the parboiled form (Witek et al., 2010; Prasert & Suwannaporn, 2009; Sittipod & Shi, 2016). The A-type starches contribute to a high amount of rapidly digestible starch and slowly digestible starch as compared to B-type starches. The shorter double helices present in A-type starches are more readily digestible, whereas B-type starches often contain high amounts of resistant starch (Jane, Wong, & McPherson, 1997). Thus, the presence of A-type starches in R may attribute to higher hydrolysis rate than the PBR. The processing of rice also impacts on starch digestibility. These results are supported by the findings of Tetens et al. (1997) who observed low starch digestibility rate in parboiled rice; and Rashmi and Urooj (2003) who found that steamed rice creates more resistant starch than boiled or pressure cooked.

For the unmilled (0 s) processed rice test group, at same time interval of hydrolysis, the rate of glucose liberation increased with increase in time (enzyme and substrate interaction). Here, the liberation rate was lowest in Fe-1a and was highest in Fe-4a (Fig. 4a). In case of FA fortified group (0 s) also, the glucose liberation rate increased with time, where the rate of PBR-a was higher than the FA fortified samples (Fig. 5a). The lower hydrolysis rate in the unmilled parboiled rice samples might be due to the physical barrier of bran, which thus prevents the enzymes to act on the starch substrate (Panlasigui & Thompson, 2006). It also might be due to the presence of fats and protein interaction with starch in the bran portion, as the presence of even small amounts of protein influences starch digestibility and functional properties in cereals (Ezeogu, Duodu, Emmanbux, & Taylor, 2008).

Among the 30 s and 60 s milled Fe fortified test group, the hydrolysis rate was more for PBR-b and Fe-2c, respectively (Fig. 4b and Fig. 4c). It was also observed that the hydrolysis rate for Fe-2c was somewhat in nearby range to PBR-c. In case of FA fortified 30 s milled samples
(Fig. 5b), the variation in glucose liberation rates were more compared to FA fortified 0 s milled samples. Whereas, for 60 s milled samples (Fig. 5c), the rates increased steadily up to 30 min, after which variation in rates were observed.

There were variations in the rate of glucose liberation between the milled and unmilled counterparts. The average HI of processed rice showed a positive correlation for Fe fortified \((R=0.86)\) and FA fortified \((R=0.99)\) with the milling time. The HI of R-c was highest, and in the processed groups, the 60 s milled samples showed higher HI. There was not much significant difference \((p<0.05)\) in the HI among PBR-a and Fe fortified samples (Fig. 4d), except in between Fe-2a and Fe-5a in 0 s milled, and PBR-b and Fe-5b in 30 s milled. The HI were not significantly different \((p<0.05)\) between the PBR-a and FA fortified 0 s milled samples (Fig. 5d), except in between FA-1a and FA-3a. The GI was found to be highest for the R-c rice (Table S1), and nearly similar GI \((83\pm5)\) was reported for white rice by Wolever et al. \((1986)\). The variations in GI may be attributed to variation in processing, compositional and accompaniment factors (Kaur, Ranawana, & Henry, \(2016)\). Except for Fe-5a, Fe-2b and Fe-2c, the estimated HI (Fig 4d) and GI (Table S1) of PBR were slightly higher than the Fe fortified rice. Similarly, the HI (Fig. 5d) and GI (Table S1) of PBR were slightly higher than the FA fortified rice, except for FA-1a, FA-1b, FA-2b and FA-4b. The lower GI in the fortified rice might be due to molecular conformational changes in starch, resulting due to Fe starch-complex (Łabanowska, Kurdziel, Bidzińska, Fortuna, Pietrzyk, Przetaczek-Rożnowska, & Rożnowski, \(2013)\) and FA-starch ester (Borah, Rappolt, Duary, & Sarkar, \(2019)\) formation, which incurred hindrances against digestive enzymes towards active sites.

In support to our finding, Guha, Ali, and Bhattacharya \(1997)\) reported a decrease in starch digestibility for rice samples extruded at 120°C as compared to 80 – 100°C, showing more
reduction in starch hydrolysis in more heat treated samples. However, our results contradict with
the findings of Bravo et al. (1998) who reported that the availability of starch in the cooked form
is more susceptible by enzymes than the unprocessed one, which results in higher percentage of
starch digestibility in cooked form. The decrease in starch hydrolysis rate in the parboiled rice
may also probably be associated with increase in steric hindrances due to branching or
reassociation of the parboiled rice starch, which causes more mass transfer resistances. This
affects enzymatic action because of mass transfer limitation. Our observations are in support of
Sanromán, Murado, and Lema (1996), who observed that when the substrate concentration of
enzyme increases, the branching itself increases the steric hindrances. Even formation of
amylose-lipid complexation, starch protein interaction, and limited water availability are reported
to lower the values of starch digestibilities (Guha et al., 1997). The resistant starch formation is
also greatly influenced by the extent of retrogradation. Frei et al. (2003) and Hu, Zhao, Duan,
Linlin, and Wu (2004) observed that retrograded amylose is resistant towards enzymatic
hydrolysis. The range of GI estimated in the present study for parboiled rice by in vitro assay is
nearly similar to the report of Wolever et al. (1986), who found the GI of 67±5 for white
parboiled rice, 65±5 for instant rice, and 54±4 for parboiled rice. Thus, it was observed that the
estimated GI of unfortified and fortified parboiled rice samples are under the marginally low to
medium/intermediate (56–69) range.

4. Conclusion

Controlled brown rice parboiling method as an alternative method for production of parboiled
ready-to-eat rice (komal chawal) was investigated as a potential to implement micronutrient
fortification. The chosen rice variety of chokuwa exhibited the characteristics of reduced cooking
requirement when parboiled. The iron and folic acid fortified rice resulted in a good amount of
bio-accessibility and bioavailability, and lower rate of starch hydrolysis. Marginally low to
intermediate range of GI values were also observed for the fortified rice. Thus, this product can
be considered as a value added product to improve micronutrient deficiency and has the potential
to be a disaster food for people in geographical locations which are prone to natural calamities.

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6. Conflict of interest

All the authors declare that there is no any conflict of interest.

7. References

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Figure 1. Percent (%) bioaccessibility of (a) Fe and (b) FA in the raw, unfortified and fortified parboiled rice. Different superscripted alphabets along the bars represent significant difference (p<0.05).

Figure 2. Percent (%) absorption of bioavailable (a) Fe and (b) FA in the unfortified and fortified parboiled rice. Different superscripted alphabets along the bars represent significant difference (p<0.05).

Figure 3. Relationship between bioaccessible and % absorption of bioavailable (a) Fe and (b) FA in the fortified parboiled rice samples

Figure 4. Glucose response kinetics of (a) 0 s milled, (b) 30 s milled, (c) 60 s milled and (d) estimated HI of raw, unfortified and Fe fortified parboiled rice samples. Different superscripted alphabets along the bars represent significant difference (p<0.05).

Figure 5. Glucose response kinetics of (a) 0 s milled, (b) 30 s milled, (c) 60 s milled and (d) estimated HI of raw, unfortified and FA fortified parboiled rice samples. Different superscripted alphabets along the bars represent significant difference (p<0.05).

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Table 1. Fortification concentrations range of Fe and FA and coding of samples

Supplementary figure:

Figure. S1. Bioaccessible form of (a) iron (mg/100g rice) and (b) folic acid (μg/g). Different superscripted alphabets along the bars represent significant difference (p<0.05).

Supplementary table:

Table-S1. Estimated glycemic index (GI) of milled and unmilled of raw, unfortified and fortified parboiled rice samples