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*Characterization of a novel **folic acid-fortified ready-to-eat (RTE)** parboiled
rice*

Elizabeth D. Wahengbam^{*1}, Brian D. Green², Manuj K. Hazarika¹

^{*1,1}Department of Food Engineering and Technology, Tezpur University, Assam, 784028

²[Institute for Global Food Security](#), School of Biological Sciences, Queen's University
Belfast, BT9 5HN, UK

***Corresponding author:**

Elizabeth Devi Wahengbam

Tezpur University, Napaam, Tezpur, Assam. PIN - 784 028 (India)

Fax : +91 3712 267005

Phone : +91 7002942217 (Mobile)

wahengbam.elizabeth@gmail.com

Abstract

Background and objectives: Effectiveness of folic acid (FA) fortification in a low amylose rice using the brown rice parboiling method was investigated. The unfortified form of the product resembles a traditional product named *komal chawal*, which require no cooking, or little cooking with shortened preparation time for consumption. For FA fortification, while conducting the parboiling process, brown rice was soaked in aqueous solutions of FA at five different concentrations (0.25-4.0 g/L), before the steaming step, to obtain products with five different levels of fortification. The concentrations of fortificant in the FA-fortified rice, milled for 0 s, 30 s, and 60 s, were assessed and the effects of FA fortification on the physico-chemical characteristics of the product were evaluated by comparing with the unfortified parboiled rice.

Findings: Fortification elevated the total FA content in uncooked rice up to 1090 $\mu\text{g/g}$, corresponding to a FA concentration in soak water of 4.0 g/l. In terms of the appearance, the product yellowness values increased with the product FA concentrations. After rehydration at 60°C for 25 min, the hardness values of all the FA-fortified parboiled rice samples (unmilled and milled) were in the range of 152-172 g which is the range of hardness value of cooked form of the rice. Chewiness and springiness values were higher in fortified samples. Pasting properties of fortified rice samples were higher than the unfortified samples. The Fourier Transform Infrared Spectroscopy (FTIR) revealed the O-H bond stretching at band range of 3000 - 3600 cm^{-1} , and a very small peak in the band ranged from 1740-1750 cm^{-1} contributed to C=O bond stretching. The FA-fortified uncooked parboiled rice showed a V-type diffraction pattern.

Conclusions: FA-fortified ready-to-eat parboiled rice (*komal chawal*), produced by brown rice parboiling method, has favorable textural and physico-chemical properties, with some yellowing effect of fortification noted at higher fortification levels with minimum effect on cooking characteristics. Hence, this procedure can find application in providing nutritional support for vulnerable people as a ready-to-eat form of low amylose FA-fortified parboiled rice during parboiling.

Significance and novelty: The approach is a pragmatic way to obtain FA-fortified ready-to-eat rice or rice with reduced cooking time to combat FA deficiency among people.

KEYWORDS

folic acid fortification, brown rice parboiling, ready-to-eat, color, pasting properties, texture analysis, FTIR

1. INTRODUCTION

More than two billion people are affected by micronutrient deficiencies. About 30% of the world's populations are anaemic due to iron deficiency, followed by folate deficiency (Haidar, 2010). Particularly, folic acid (FA) is involved in several roles such as in nervous tissue, nucleic acid synthesis and protein metabolism. Apart from such roles, homocysteinemia and neural tube defects (NTD) are the two important diseases caused due to folate deficiency (Krishnaswamy & Nair, 2001; Choi & Manson, 2002).

To improve the micronutrients in rice grains efforts are on by the researchers. Fortification of micronutrients in staple foods has been on focus as they can offer as a carrier to eliminate micronutrient deficiency although they are not a good source of micronutrients of their own. Methods of fortification like supplementation, bio-fortification, coating, extrusion technologies and dusting methods are not considered as cost-effective approaches for delivering nutrients to the mass population. Fortification by the method of fortification during parboiling is preferred over such methods (Fukai, Godwin, Rerkasem, & Huang, 2008; Steiger, 2014).

In South Asian countries like India and Bangladesh which use rice as the staple food are known to be hugely affected by micronutrient deficiency. While about 15% of parboiled rice form is consumed out of the world's milled rice production (Bhattacharya, 2004), India and Bangladesh are the largest consumer of parboiled rice as a staple food (Hettiarachchi, Hilmers,

Liyanage, & Abrams, 2004). Hence the method fortification of rice by parboiling is a more potential option for this region. There are reports on the FA fortification in the whole grain parboiled rice, brown parboiled rice, and bread (Öhrvik, Öhrvik, Tallkvist, & Witthöft, 2010; Kam, Arcot, & Adesina, 2012^a). The brown rice parboiling facilitates quick water uptake during the soaking step due to the removal of the husk which in turn reduces the processing time as well as discoloration, low bulk handling, faster and cheaper processing, and reduced operation cost (Kar et al. 1999; Oli et al. 2016).

Therefore, in the present study, FA fortification was applied to a low amylose rice variety called *chokuwa* by brown rice parboiling method. The ready-to-eat characteristics of the parboiled brown rice from this variety is reported in Wahengbam and Hazarika. The present work aimed to increase FA concentration in the rice to produce ready-to-eat FA-fortified parboiled brown rice and characterize the product for its potential as a carrier of FA for benefits of folate deficient population of the region.

2. MATERIALS AND METHODS

2.1 Materials

A low amylose variety of paddy named *chokuwa* was obtained from the nearby local farm of Tezpur, Assam, India. Then dehushing was done, by using sheller (RTE-07, A-GRAIN, India) to get the brown rice and then stored in an air tight container in refrigerated condition.

2.2 Folic acid (FA) fortification through brown rice parboiling process

Approximately 100 g of dehusked *chokuwa* brown rice was fortified with five different ranges of folic acid ($C_{19}H_{19}N_7O_6$, Sigma Aldrich) in a 1:2 ratio of rice to water (Table 1). After draining the excess water, followed the steaming (at 15 psi for 10 min) and drying (at 40°C up to 12 %

moisture) steps. Similar processing steps are followed for unfortified parboiled brown rice except the addition of FA. Further, the fortified and unfortified parboiled rice were milled for 30 s and 60 s by using the polisher (RTE-08, A-GRAIN, India).

2.3 Coding of samples

Coding were made as FA-1 to FA-5 with increasing five different range of FA concentration (Table 1). **Three different duration of milling were considered to quantify the effects of milling.** The unfortified parboiled brown rice (PBR) are coded without fortification concentration range with a suffix of polishing time only, for example, PBR-a, PBR-b and PBR-c (Table 1).

2.4 Determination of total folic acid content

Extraction and purification (n=3) were carried out by following Kam et al. (2012a) prior to HPLC analysis in a HPLC instrument (Waters 2695, USA) using a photodiode array detector (Waters-2996), monitored at 280 nm. The separation was performed using a Luna 5u C18(2) column (150 x 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). The program followed was 0 to 5 min (1% B); 5 to 20 min (1% - 25% B); 20 to 21min (25% - 80% B); 21 to 25 min (80% B); 25 to 26 min (80% to 1% B); and 26 to 30 min (1% B). The run time was 30 min between injections (10 µl volume). Based on the retention time of the folic acid standard at particular absorption spectrum peak was identified. To ensure the data integrity HPLC calibration was done on a daily basis.

2.5 The percent (%) uptake of folic acid

The % uptake of FA in the fortified parboiled rice was calculated by following equation 1 (Kam, Arcot, & Ward, 2012^b)

$$Uptake (\%) = (FA_{total}/FA_{added}) \times 100 \quad (1)$$

where FA_{total} is the analysed total folic acid amount in the uncooked fortified parboiled rice; and, FA_{added} is the initial amount of folic acid added to the soaking water.

2.6 Percent (%) retention of folic acid

To have an estimate of loss of fortificant upon rehydration at boiling water, open cooking (100°C) was carried out by following Wahengbam & Hazarika, (2018), and assessed for folic acid retention. Percent retention of FA was determined by following equation 2 (Kam et al., 2012^b)

$$Retention (\%) = (FA_c/FA_{total}) \times 100 \quad (2)$$

where FA_c is the analysed folic acid amount in the cooked fortified parboiled rice.

2.7 Changes in grain color

The lightness (L^*), yellowness (b^*) and redness (a^*) were measured by using HunterLab (UltraScan VIS), and the changes in the total color difference (ΔE) and chroma (C^*) values are derived from $L^*a^*b^*$ values. Five samples were examined each time.

2.8 Wide angle X-ray diffraction (XRD) pattern

The XRD pattern was measured by using a X-ray diffractometer (Bruker Axs, Germany). The spectra were scanned over a diffraction angle (2θ) range of 10–40° at a step size of 0.05°, with Cu K α value of 1.54 Å (λ) operated at 30 kV. The peak centre, peak area & Full Width Half Maximum (FWHM) were determined using software (OriginPro 8.5). The interplanar distance or ‘d-spacing’, size of crystallites (γ) and mean percent (%) crystallinity were determined by following Wahengbam & Hazarika, (2018).

2.9 Pasting properties

The pasting parameters of rice flour suspensions (12 % w/w; 28 g total weight) were determined by following Klein et al., (2013).

2.10 Textural parameters of cooked and rehydrated rice

The cooking and rehydration procedure was performed by following the method of Wahengbam & Hazarika, (2018). The textural properties were determined (n=9) for cooked and rehydrated (60°C for 10, 15, 20 and 25 min) samples using a texture measuring instrument (TA-HD plus, Stable Micro Systems, UK), two-cycle compression test was used by following the procedure of Dutta & Mahanta, (2014).

2.11 Fourier-transform infrared (FTIR) spectroscopy

The frequency and the intensity of the peaks of the FTIR bands finds the changes in the structure of the starches. The thin pellet of sample was prepared after mixing and pressing the dried rice flour and desiccated KBr powder. The FTIR spectrometer (Frontier IR, Perkin Elmer) equipped with KBr optics and a DTGS detector, was operated with a resolution of 4.0 cm⁻¹, the infrared absorption spectra were obtained at scanning range of 4000–400 cm⁻¹.

2.12 Software for statistical analysis

Both Microsoft Excel 2010 and SPSS 20 (IBM SPSS) were used in this work.

3. RESULTS AND DISCUSSION

3.1 Determination of total FA, % uptake and % retention

The total folic acid (FA) content, % uptake and retention in the FA-fortified rice is given in Table 1. The FA concentration in the unfortified parboiled rice could not be quantified. It was below the quantification limits (0.40 µg) of the HPLC. A significant increased in total FA content was observed from 79 to 1090 µg/g rice with an increase in FA fortification

concentrations, indicating the uptake of FA due to the addition of the FA. The uptake of FA might be preceded through the diffusion process of the folate anions from the solution to the aleurone layer where adsorption takes place (Kam et al., 2012^a).

A successive reduction of FA concentration was evident (Table 1) with an increased in milling time. It showed the migration of fortificant from the soaking water, through the outer layer (aleurone layer) into the inner layer of the endosperm. The loss of residual FA concentration between 0 s and 30 s milling varied from 2.13% to 24.98%, and 2.64% to 38.89% for between 0 s and 60 s, with an average loss of 10.24% and 15.04%, respectively. The loss of FA concentration between 30 s and 60 s was up to 14% with an average of nearly 5%. High reduction was observed due to increased in milling duration. It showed that the FA was mostly distributed on the outer portion (aleurone layer), thus average % loss in 30 s was high compared to 30 to 60 s milling. It also possibly indicated that beyond the outer aleurone layer, the migration of fortificant was strongly bonded to the hydrolysed carbohydrate molecules of the rice (Kam et al., 2012^a).

The average % uptake in the first three initial FA-fortified parboiled rice (FA-1 to FA-3) were comparatively higher than the highest concentrated FA-fortified samples (FA-4 to FA-5). The percent (%) retention of FA in the cooked fortified samples was varied from 55 to 96%. Retention increased with an increase in fortificant concentration, and reduced with an increase in milling time. The FA content in the unmilled and cooked FA-fortified rice was between 98 and 673 µg/g rice. The loss of FA in the cooked FA-fortified parboiled rice in between 0 s to 30 s, 30 s to 60 s, & 0 s to 60 s milling were varied from 3.65 to 23.49%, 11.38 to 25.56% and 4.37 to 35.50 %, respectively. The % retention of FA-4a and FA-5a fortified cooked rice samples was

comparatively less than the FA-1a to FFA-3a samples. It indicated that, while cooking the loss of FA was marginally higher for unmilled samples.

3.2 Changes in color values

The changes in color values are presented in the Table 2. By increasing the FA fortification concentration, the L^* (64.54–69.93) and a^* (-1.32– -0.64) values of unmilled (0 s) samples were decreased, while b^* (23.93–25.77) value increased with concentration. On the other hand, with increased in milling time, the L^* value slightly increases, however, the a^* (redness) and b^* (yellowness) values decreased. In case of FA-1 and FA-2 group of FA-fortified rice, the b^* values were less significant ($p \leq 0.05$) to that of unfortified parboiled brown rice (PBR). Nearly similar changes in L^* , a^* , and b^* values were reported by Kam et al., (2012^b). The yellowness values was contributed due to the addition of FA, and also the pigment diffusion from the bran. The progressive yellow intensity corresponding to the increasing concentration levels was significantly different among FA-3, FA-4 and FA-5 samples. The total color difference (ΔE) of unmilled FA-fortified samples were between 12.06 and 17.52, which was affected by milling time. The chroma (C^*) values of 30 s and 60 s milled samples of FA-1 and PBR were less significant ($p \leq 0.05$).

3.3 X-ray diffraction patterns

The XRD pattern of unmilled (0 s) unfortified and FA-fortified parboiled rice flour samples is shown in Figure 1, and its XRD peak centre (2θ), % crystallinity, FWHM, d spacing and crystallites size are given in the Table S1 of the supplementary file. The unfortified and FA-fortified parboiled rice showed the V-type diffraction pattern and a nearly similar pattern was reported in instant rice by Prasert & Suwannaporn, (2009). The caused of such pattern is due to

the destruction of the crystalline structure of the starch granules and formation of amylose–lipid complexes during hydrothermal process (Shih et al., 2007). The % crystallinities of unfortified and fortified parboiled rice were less significant differences. The changes in FWHM ($^{\circ}$), d spacing and crystallites size among unfortified and fortified samples are depends on peak size. The d spacing (nm) of the first peaks were slightly more than the second peaks. The crystallites size (nm) of unfortified and fortified parboiled rice was less significant except for FA-2a, FA-3a and FA-5a. The slight changes in the crystallites size of unfortified and FA-fortified samples might be due to gelatinization and retrogradation process, in addition the penetration of FA, might also slightly modified structurally.

3.4 Pasting properties of rice flour

Pasting involved a gelatinization phenomenon of granular starch swelling, with emission of molecular components and eventually total degradation of the granules. The pasting properties of unfortified and FA-fortified parboiled rice for unmilled and milled (60 s) are shown in Figure S1a and b of supplementary file, respectively. The peak viscosity (PV) of unmilled FA-fortified rice (719 to 950 cP) increases with an increase in FA concentration (Figure S1(a)). However, for milled counterparts (Figure S1(b)) it was not in the increasing order with concentrations. This may be due to variation in the removal of bran portion during milling despite of fixed milling time. The unmilled, unfortified parboiled rice (PBR-a), showed lowest PV (227 cP), HPV (225 cP), BV (2 cP), FV (368 cP) and SBt (143 cP). Similar patterns in instant rice was reported by Prasert & Suwannaporn, (2009). Despite the similar processing condition, the PV, HPV, BV, CPV, SB and SBt in the FA-fortified rice were more than the unfortified parboiled brown rice (PBR). The milled rice samples had higher PV, HPV, FV, and SBt, similarly reported by Perdon, Siebenmorgen, Mauromoustakos, Griffin, & Johnson, (2001). This changed might be due to the

removal of the bran. Final viscosity, slightly increased with concentration among the fortified samples. It indicated the ability to form a viscous paste after cooking and cooling. The SB was increased with an increase in milling time, which indicated the more tendency of the dough to undergo retrogradation (Sanni, Adebowale, Olayiwola, & Maziya-Dixon, 2008). The SB of unmilled was low as compared to milled counterparts. The negligible BD and a linear rise in the viscosity of processed rice might be attributed to leaching of the short linear molecular chains. This caused thickening phenomenon suggesting its suitability for specific uses.

3.5 Textural properties

The textural attributes of the cooked and rehydrated (soaked at 60°C) FA-fortified parboiled rice samples were compared with the unfortified parboiled rice samples (Figure S2 (a-d)). The hardness values of cooked rice were between 152 and 172 g. The hardness values of 10 min soaked were higher than the 15-25 min soaked samples (Figure S2(a)). The range of 15 to 25 min soaked hardness values of 60 s milled FA-fortified parboiled rice (FA-1c – FA-5c) samples were between 400 and 144 g, respectively. The milled samples hardness values were comparatively lower than the unmilled counterparts. It might be due to the removal of the bran layer which lead to faster rehydration. The hardness values of all the 25 min soaked FA-fortified parboiled rice samples (unmilled and milled) were close to that of cooked rice counterparts. Likewise, the hardness values of most of the 20 min soaked unfortified PBR samples were nearly or less than or equal to that of cooked rice.

The adhesiveness of cooked rice samples were less than the soaked counterparts (Figure S2(b)). Adhesiveness of most of the 10-15 min soaked samples was higher compared to 20-25 min soaked samples. A significant difference ($p \leq 0.05$) was observed among the rice samples. It indicated that more energy is required to overcome the sticky forces between the rice and probe.

The chewiness of 20-25 min soaked, and cooked rice samples were less than to 10-15 min soaked counterparts (Figure S2(c)). It showed that more energy was required to chew the less rehydrated samples. The springiness values of 20-25 min soaked PBR and FA-fortified rice samples were nearly similar to that of cooked form (Figure S2(d)). It showed that samples have the ability to returned to its original shape after the deformation. Texture is a complex-multidimensional characteristics, although the hardness and adhesiveness are the important properties in deciding the palatability of cooked rice (Zhou, Robards, Helliwell, & Blanchard, 2002). The hardness being the most important characteristics of texture. The hardness values of cooked rice were similar to that of 20-25 min rehydrated unfortified and FA-fortified parboiled rice samples. This condition might be considered as the ready-to-eat form of FA-fortified rice.

3.6 Changes in the structure by FTIR spectroscopy

FTIR is the vibrational spectroscopic techniques that responds directly to interactions with the molecular structure of the food sample. It was used to measure the quality of food by investigating process-induced changes by analyzing the frequency and the intensity of the peaks (Scotter, 1997). Thus, this technique was used to monitor changes in the structure of the raw rice starches after FA fortification during the hydrothermal treatment process. The FTIR spectrum of 60 s milled and unmilled samples of raw and folic acid fortified parboiled rice is shown in Figure 2. The strong broad peak was observed at 3388, 3404, 3415, 3399, 3412 and 3390 cm^{-1} for R-a, FA-1a, FA-2a, FA-3a, FA-4a and FA-5a, respectively. In case of 60 s milled samples, the broad peak was observed at 3388, 3412, 3410, 3403, 3397, and 3390 cm^{-1} for R-c, FA-1c, FA-2c, FA-3c, FA-4c and FA-5c, respectively. These broad peaks are due to stretching of the O-H functional group (3000-3600 cm^{-1}). A sharp, strong ('s' in raw) and medium ('m' in FA-fortified) peaks were observed in both 60 s milled and unmilled (0 s) samples at 2926.34, 2922

(s); 2926, 2921; 2923, 2922; 2921, 2922; 2928, 2928; 2927 and 2922 (m) cm^{-1} for raw; FA-1; FA-2; FA-3; FA-4 and FA-5 group (both 60 s and unmilled) rice, respectively. These sharp peaks might attributed to stretching of C-H bond (Kizil, Irudayaraj, & Seetharaman, 2002). A very small peak was observed at 2849-2860 cm^{-1} except for 60 s milled, raw rice sample. These small peaks are found at 2849 (FA-1c), 2852 (R-a & FA-4a), 2852 (FA-4a), 2853 (FA-2c & 3c), 2856 (FA-5c), 2858 (FA-1a, 3a & 5a), 2859 (FA-2a), and 2860 (FA-4c) cm^{-1} . The changes in the ratio of the band intensities of these small and sharp peaks pattern were observed with minor changes in the intensities for all the samples which are responsible for symmetric and asymmetric stretch of H-C-H (Dutta & Mahanta, 2012). Very small peaks were found in the band ranged from 1740-1750 cm^{-1} . It contributed to C=O bond stretching. A strong peaks were found in all samples at band ranged 1648 to 1661 cm^{-1} , with minor changes in the band intensities of folic acid fortified parboiled rice which also contributed to C=O bond stretching. The sensible changes in the frequency of the absorption band were observed. It showed a slight change in molecular level among processed samples. The changes in band shift in the untreated raw (R) sample are more strong than the FA-fortified sample.

4. CONCLUSIONS

The FA fortification during the production of parboiled brown rice *komal chawal* provides a good amounts of folic acid. The product has favorable textural and physico-chemical properties, but slight yellowness was noted at high fortification levels. Less significant differences were found in the % crystallinity of unfortified and FA-fortified parboiled rice samples. The pasting properties of FA-fortified parboiled rice was more than unfortified parboiled rice. The rehydration condition for the ready-to-eat FA-fortified rice was 20-25 min at 60°C. Further

research is required for quantifying the bio-accessible and bioavailable forms of FA from such fortified rice in the human diet.

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