

# Micronutrients Fortification of Rice by Parboiling: Lab Scale and Pilot Scale Studies

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## Abstract

Deficiency diseases due to micronutrient insufficiency occur worldwide, especially in developing countries. Folic acid deficiency causes neural tube defects (NTD) and affects the central nervous system in 1:1000 births leading to anencephaly and spina bifida. Anaemia, a public health problem is caused by iron deficiency in 50% of the cases. Sub-clinical Vitamin A deficiency (VAD) causes morbidity and mortality, especially among young children and pregnant women. In young children, it can cause xerophthalmia and keratomalacia and lead to blindness or limited growth and weakening of the immune system, thereby increasing the risk of death. This study investigates the efficiency of using parboiling as a processing technique to fortify rice with folic acid,  $\beta$ -carotene and iron. Brown rice was parboiled by soaking with the micronutrients at the optimum conditions (70°C for 2 hours), steaming the soaked rice at 100°C for 1 hour and air drying until the moisture dropped to  $10 \pm 2\%$  wb. Retention of these nutrients was assessed by analysis in the dried rice after milling (0 s, 60 s and 120 s) and in the cooked rice. The fortification was scaled up using 2.5 kg rice by parboiling with the highest concentration of the micronutrient mixture. The experimental process was compared with the conventional parboiling process for which the retention of the micronutrients was analysed. Retention of the nutrients in rice reduced during milling and cooking decreased by 10-15% (folic acid); 2-8% ( $\beta$ -carotene) and 4-5% (iron). Fortification of rice using parboiling is feasible. The laboratory scale method correlated well with the pilot scale.

**Keywords:** Parboiling; Fortification; Folic acid; Iron;  $\beta$ -Carotene.

## Introduction

### Vitamin A deficiency

Micronutrient deficiencies are common in developing countries such as Asia, Africa and the Pacific. Vitamin A deficiency can lead to diseases such as xerophthalmia, Bitot's spot, conjunctival xerosis, corneal lesion, xerosis, ulcer, scars and keratomalacia. All of these deficiency conditions can lead to poor vision or total blindness if left [1-6]. For example, during the year 2000, in India 5,049,139 people were affected out of a total population of 841,523,272 [7].

### Iron deficiency

About one third of the world population suffers from iron deficiency anaemia (IDA). India continues to be one of the countries with a high prevalence. National Health Family Survey (NHFS, in 1998-1999) revealed that about 70-80% of children, 70% of pregnant women and 24% of adult men were suffering from IDA [8].

### Folic acid deficiency

Folic acid is one of the important B-complex vitamins essential for biosynthesis of DNA, and for purine and amino acid inter-conversions. It cannot be synthesized by the body and therefore it needs to be obtained from dietary sources. Folic acid deficiency leads to cardiovascular diseases, major depression, schizophrenia, Alzheimer's disease, and some carcinomas such as colorectal, uterine, cervical, lung and oesophageal. Various studies and randomised trials over the last three decades have shown that adequate intake of folate during early pregnancy reduces the risk of abnormalities in early embryonic brain development and specifically the risk of malformations of the embryonic brain/spinal cord, collectively referred to as NTDs [9-14]. Fortification of wheat flour to overcome folic acid deficiency diseases has been proven effective in many developed countries. Fortification of folic acid (100 to 150  $\mu\text{g}/\text{day}$ ) of cereal grain products resulted in decrease in NTDs in the United States and Canada [15,16]. Pachón, et al. state that with the widespread consumption of wheat flour-based

products and advances with technology in the milling industry, folic acid fortification should be adapted as a public health initiative in Europe [17]. In Australia, 2 per thousand live births are affected by NTDs attributed to folic acid deficiency. As a result, fortification of bread making flour with folic acid has been mandatory in Australia since September 2009 [18].

### Medium for fortification

Rice is the staple food of Asia and parts of the Pacific. Over 90% of the world's rice is produced and consumed in the Asia-Pacific region. Therefore, it would be a good choice to fortify rice with Vitamin A, folic acid and iron as a comprehensive fortification. For successful fortification, the nutrient should be aesthetically acceptable when mixed with the appropriate vehicle; stable under normal conditions of storage; and easily bioavailable when consumed [19].

### Parboiling as a means for Fortification

The parboiling technique of rice is a hydrothermal process resulting in irreversible swelling of starch during which the crystalline form of starch in rice is converted to the amorphous form. It can be achieved through soaking in hot water followed by steaming, drying and milling. After parboiling, gelatinized starch and protein bodies occupy the empty spaces in the rice endosperm thereby giving better toughness and hardness to the rice kernel. The kernel becomes translucent and

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offers great resistance to breakage during milling. During parboiling, dextrinisation of starch and destruction of lipase can occur, thereby improving the shelf-life of rice. Parboiled rice has better nutritional properties than white rice because the nutrients from the bran migrate towards the endosperm during parboiling and therefore there is less loss of nutrients during milling [20-22]. Previously, rice fortification using parboiling was feasible for single nutrient fortification: iron, zinc [23] and folic acid [24]. In the present study fortification of rice by parboiling with folic acid, iron and  $\beta$ -carotene was successfully implemented by soaking the rice at 70°C for 2 hours and steaming at 100°C for 1 hour. The parboiled rice was then air dried until the moisture content dropped to 10-12%. This experimental condition was compared to conventional parboiling on a pilot scale to assess whether large scale processing and fortification is practically possible.

## Materials and Methods

### Brown rice samples

Dehusked brown rice of a known long grain variety (Pusa 1121) was purchased in large quantities from a local supermarket in Sydney, Australia and stored at 11°C. Prior to analysis the rice was cleaned from dust and broken grains using a rubber roll dehuller (purchased from Satake, Japan).

### Fortificants used for parboiling

Food grade folic acid was purchased from DSM, Australia. Lucarotin 10 CWD S Y which has  $\beta$ -carotene encapsulated in a proteinaceous matrix was purchased from BASF Company, Germany. Sodium EDTA iron (III) was purchased from Ferrazone, The Netherlands.

### Parboiling Procedure

Graded brown rice was soaked in water in the ratio of 1:2 with the micronutrients at 70°C for 2 hours. The mass of micronutrients added per 300 g rice (per 600 mL water) is listed in Table 1. In order to provide at least half of the recommended intakes for the three nutrients, condition A was chosen as the optimum condition to prepare the fortified premix. The nutrient concentrations in A were appropriate for dilution of the premix with of unfortified rice (1:10) before sale.

The rice was soaked with the designated conditions at 70°C for 2 hours after which the soaking water was discarded and the rice was transferred into a steaming box and steamed at atmospheric pressure (100°C) for 1 hour to induce gelatinization. The samples were spread and air dried at room temperature until the moisture content dropped to 10  $\pm$  2%. Each condition was replicated and a control sample was also prepared by parboiling the rice without any micronutrients in the soaking water.

### Parboiling procedure for Scale-up Studies

Cleaned, dehusked brown rice (2.5 kg) was soaked in water in the ratio 1:2 with the micronutrient solution at 70°C for 2 hours followed by steaming at 100°C for 1 hour (experimental condition). This treatment was compared with soaking at 70°C for 4 hours with 15 minutes steaming at 100°C followed by tempering for 45 min in an air-tight

Condition	Folic acid	-Carotene	Iron
A (mg)	150	62.5	25
B (mg)	300	125	50
C (mg)	600	250	100
D (mg)	1200	500	200

**Table 1:** Mass of micronutrients added per 300 g of rice for Parboiling.

container (conventional industrial process). The samples were spread and air dried at room temperature until the moisture content dropped to 10  $\pm$  2%. Each condition was replicated and a control sample was also prepared by parboiling the rice without any micronutrients in soaking water. The nutrients added were 1.2 g folic acid, 500 mg  $\beta$ -carotene, 200 mg iron in 5 L water.

### Milling of parboiled rice

For each sample, 100 g of parboiled brown rice was milled in a laboratory scale grain mill (Satake Test Grain Mill, Japan) for 60 and 120 seconds to yield white rice. The corresponding degree of milling which was measured by loss in mass was ~6 and 11% respectively.

### Cooking of parboiled rice

For cooking the parboiled fortified rice, 10 g of each sample was cooked with 25 mL of water in a 100 mL beaker and then it was mashed and homogenized for analysis. The rice that was milled for 120 s was used for cooking because the whiter coloured rice was popular with the consumers.

### Moisture content in fortified rice

The moisture content of brown rice was determined by the standard oven method [25]. Throughout soaking, tea infusers containing 20 g rice were removed in triplicates from the water bath at regular intervals (i.e. 0, 30, 60, 90, 120 minutes). ~20 g samples were placed in moisture dishes, weighed and dried in vacuum oven at 70°C for 24 h. The dishes were taken from the oven and cooled for 3 h in a desiccator. Moisture content was expressed on a wet basis (w.b.).

### Sample extraction and analysis for the micronutrients

The milled rice and brown rice were analysed for each micronutrient in duplicates to determine the retention of the fortified micronutrients and also to understand the nutrient loss on milling.

### Analysis of folic acid

Folic acid analysis was done according to Pfeiffer et al. and Tamura with some modifications [26,27]. Single enzyme extraction was done with  $\alpha$ -amylase by incubation of the rice extract for 2 h at 37°C. At the end of the incubation, the enzyme was deactivated by heating the sample in boiling water bath for 3 min. The sample was cooled, centrifuged at 4000 rpm for 15 min and the supernatant was collected for analysis.

Sample purification was carried out by solid-phase extraction (SPE) on a strong anion exchange cartridge (500 mg, 3 m, Phenomenex, Australia). The cartridge was conditioned with methanol (2x2.5 mL) and water (2x2.5 mL) to activate the sorbent and remove matrix interfering components [28]. After loading an aliquot of the sample extract, the cartridge was washed (3x2.5 mL) with water and eluted under gravity with 0.1 M sodium acetate containing 10% sodium chloride and 1% ascorbic acid. The purified sample was filtered through 0.45  $\mu$ m regenerated cellulose (RC) syringe filter (Minisart RC 25, Germany) prior to HPLC analysis.

Analyses were performed using an HPLC system (model LC AD, Shimadzu Prominence, USA) consisting of an autosampler, a thermostable column compartment (maintaining the column temperature at 35°C) and a photodiode array detector (monitoring at 280 nm). The HPLC system was controlled by a computer running LC Solution Shimadzu Chromatogram Data System.

The separation of folic acid was performed by using a reversed-phase Luna C18 column, 5  $\mu$ m, 150x4.6 mm i.d. (Phenomenex, Australia) with a C18 security guard column (Phenomenex, Australia).

Folic Acid was determined by using a gradient elution program with acetonitrile and 30mM phosphate buffer (pH 2.2). The flow rate was 0.8 mL/min. The injection volume was 100 µL. The gradient program was as follows: 5% (v/v) acetonitrile maintained isocratically for the first 8 min which was then raised linearly to 24% within 23 min then returned to 5% after 5 min. The run time between injections was 40 min. The peak was identified by the retention time and absorption spectrum acquired for the peak corresponding to the external folic acid standard. HPLC calibration was done daily to ensure data integrity.

### Analysis of β-carotene

β-carotene analysis was done accordingly. The extract was dissolved in 3 mL of methanol and then passed through 0.45 µm polytetrafluoroethylene (PTFE) syringe filter (Minisart RC 25, Germany) prior to HPLC analysis. Analyses were performed using a HPLC system (model LC AD, Shimadzu Prominence, USA) consisting of an autosampler, a thermostable column compartment (maintaining the column temperature of 40°C) and a photodiode array detector (monitoring at 450 nm). The HPLC system was controlled by a computer running LC Solution Shimadzu Chromatogram Data System.

The detection of β-carotene was performed by reverse phase HPLC using Luna C18 column, 5 µm, 150x4.6 mm i.d. (Phenomenex, Australia) with a C18 security guard column (Phenomenex, Australia). β-carotene was determined using isocratic elution with methanol and acetonitrile in the ratio 40:60. The run time between injections was 30 min. Peak identification was by the retention time and absorption spectrum acquired for the peak corresponding to the external folic acid standard. HPLC calibration was done on a daily basis to ensure data integrity.

### Analysis of Iron

Approximately 0.5 g of ground rice sample was digested overnight with 10 mL of 70% nitric acid. The digested sample was heated on a heating mantle until it produced brown fumes. When the fumes subsided the samples were cooled and were treated with 2 mL of 35% hydrogen peroxide. The samples were heated again for about 5 minutes, cooled and filtered to remove particulate matter. The filtrate was made up to 25 mL and analysed using ICP-OES. Yttrium (1 ppm) was added online as internal standard to correct for the loss of analyte during sample injection [29].

The ICP-OES system (Perkin Elmer, Model: Optima DV7300 ICPOES) was run under the conditions mentioned in Table 2.

The quantification was performed using the calibration curve with iron standards (1000 ppm stock solution obtained from High-Purity Standards, South Carolina) diluted from certified 1000 mg/L iron standard that was simultaneously run alongside samples to maintain integrity of the analysis. The limit of detection was 0.05 ppm.

Radio Frequency Power	1300 watts
Plasma Gas Flow	15 L/min
Auxiliary Gas Flow	0.5 L/min
Nebulizer Gas Flow	0.70 L/min
Sample Introduction System	Burgener PEEK Mira Mist nebuliser with cyclonic spray chamber
Pump rate	0.5 mL/min
Viewing height	15 mm above load coil
View mode	Axial
Number of Replicates	3

**Table 2:** Conditions of ICP-OES instrument for Iron analysis.

### Calculation of % uptake from the fortificant during parboiling and % retention of micronutrients in rice after cooking

Uptake of the micronutrients into the rice refers to the amount of folic acid, iron and β-carotene present in the rice kernel after parboiling (after soaking, steaming and drying). The % micronutrient uptake in fortified parboiled rice (uncooked) was calculated as the ratio between the amount of the respective micronutrients present in the fortified parboiled rice kernel and the corresponding folic acid added to the soaking solution as the fortificant. Hence, the % uptake was calculated as in equation 1:

$$\% \text{Uptake} = \left( M_{\text{fort-rice}} / M_{\text{fort}} \right) \times 100 \quad (1)$$

where  $M_{\text{fort-rice}}$  is the analysed amount of nutrient in the uncooked fortified parboiled rice; and,  $M_{\text{fort}}$  is the initial amount of micronutrient added to the rice in the soaking water (Table 1).

The % retention of micronutrients refers to the mass of nutrients present in the cooked fortified parboiled rice in relation to the amount of folic acid present in the uncooked form of fortified parboiled rice. The % retention was calculated as in equation 2.

$$\% \text{Retention} = \left( M_{\text{cooked rice}} / M_{\text{fort-rice}} \right) \times 100 \quad (2)$$

where  $M_{\text{cooked rice}}$  is the analysed folic acid amount in the cooked fortified parboiled rice.

The % loss of micronutrients refers to the nutrient loss due to parboiling in the soaking solution. Nutrient loss in fortified parboiled rice (the. nutrient loss percentage) was the ratio between the amount of fortificant present in the soaking solution after parboiling and the initial amount of fortificant added to the soaking water (Equation 3)

$$\% \text{Loss} = \left( S_{\text{fort-rice}} / S_{\text{fort}} \right) \times 100 \quad (3)$$

where  $S_{\text{fort-rice}}$  is the analyzed nutrient content in the soaking solution after parboiling (mg); and  $S_{\text{fort}}$  is the initial amount of nutrient added to the soaking solution (mg).

### Data analysis

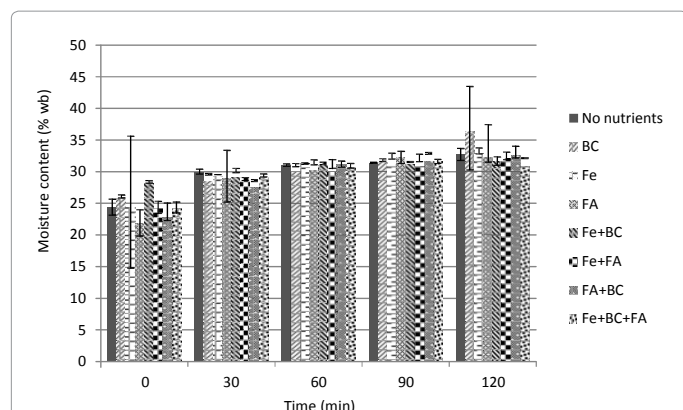
The analysis of variance was carried out by using Statplus, analytical software, AnalystSoft to detect the differences of nutrient concentrations in soaking water of brown rice before and after parboiling.  $p < 0.05$  was applied to compare the means for significance differences between the fresh and soaked samples, in terms of nutrient concentrations in different combination of nutrients added into soaking water.

## Results and Discussion

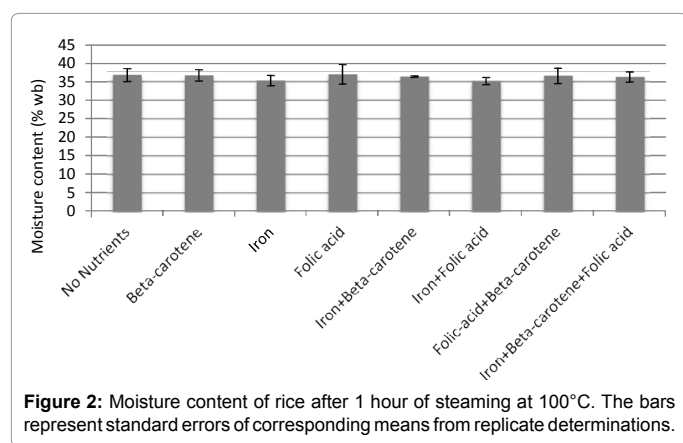
### Moisture content in fortified rice

Soaking is done to achieve quick and uniform water absorption. The grain reaches a moisture content of 30-35% in 2 to 4 hours [30], which was also observed in this study (Figure 1).

During the first 30 min soaking, the moisture content for all samples increased from ~22-28% to ~28-30%. At 60 min, the moisture content was approximately 31%. There was a further increase at 90 min for samples fortified with iron; folic acid; iron and folic acid; and folic acid and β-carotene, where the moisture content reached 32%; whereas others slightly increased. At 120 min, rice fortified with β-carotene achieved the highest moisture content of 37%, whereas with folic acid the moisture content increased the most from 22% at 0 min to 34% at 120 min. The moisture content of rice fortified with β-carotene and folic acid increased from 24% at 0 min to 33% at 120 min.



**Figure 1:** Moisture content of rice with different fortification conditions during soaking at 30 min time interval. BC:  $\beta$ -carotene; Fe: iron; FA: folic acid. The bars represent standard errors of corresponding means from triplicate determinations.



**Figure 2:** Moisture content of rice after 1 hour of steaming at 100°C. The bars represent standard errors of corresponding means from replicate determinations.

After steaming, the moisture content of all samples further increased and ranged from 35-37%. In particular, rice fortified with iron+folic acid had the lowest moisture content of 35%, while rice fortified with folic acid had the highest moisture content of 37% (Figure 2).

### Retention of micronutrients in the fortified rice and Effectiveness of Parboiling as a method of Fortification

There was no detectable folic acid and  $\beta$ -carotene found in the unfortified (control) brown rice. The unprocessed (wholegrain) brown basmati rice (used in this study) had 63  $\mu\text{g}$  of iron/g. Rice is not a natural source of folic acid or  $\beta$ -carotene; these 2 compounds were not present in the unfortified rice. Conventionally-bred rice has about 7-13 mg/kg of iron and transgenic rice has up to 37 mg/kg of iron [31]. The choice of the nutrients to be added was important as they constitute major deficiency in people's diets worldwide.

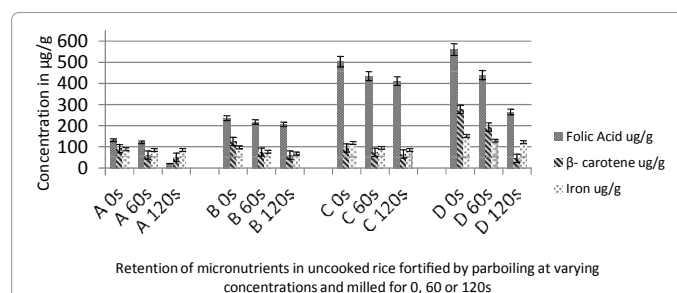
Figure 3 shows the retention of micronutrients in parboiled fortified uncooked rice. At various conditions of addition of the fortificants to the rice, folic acid showed the highest retention followed by  $\beta$ -carotene and iron. Folic acid retention was 20 to 560  $\mu\text{g}/\text{g}$  of rice. For  $\beta$ -carotene the retention was 50 to 277  $\mu\text{g}/\text{g}$  of rice and for iron the retention was 84 to 150  $\mu\text{g}/\text{g}$  of rice. With the increase in the concentration of fortificants added to the soaking water the retention of micronutrients increased. The retention analysis was performed at 3 different milling times (0 s, 60 s and 120 s). Folic acid was retained highest in the aleuronic layer of the rice which is in agreement with an earlier study [24]. In the case for  $\beta$ -carotene a similar trend was observed. The aleuronic layer

had the highest retention and with the increase in the milling time the nutrient retention decreased. The retention of iron, however, was similar in the aleurone layer and endosperm: fortification through parboiling increased the concentration of iron in the outer surface of rice although, unlike folic acid and  $\beta$ -carotene, there was very little penetration into the grain. The migration of micronutrients by inward diffusion into the endosperm during the soaking stage [32] occurs due to the difference in the moisture gradient between the rice kernels and the surrounding environment. During soaking there was a positive uptake of the nutrients into the rice as the soaking was determined to be the main step of fortification during the parboiling process.

With an increase in the soaking time it is expected that more nutrients diffuse into the rice as there is more time for the nutrients to be dispersed in the soaking solution and this can result in higher uptake of the micronutrients in the rice [33]. Folic acid showed similar retention in lower concentration as that mentioned in the work done by Kam, et al. [24]. Higher concentrations of folic acid along with the other 2 micronutrients led to higher retention. Parboiling has been previously employed to fortify rice with iron, zinc and folic acid as single nutrients [24,34,35], but not in combination. Kinetics of folic acid fortification in parboiled rice was studied by Kam et al. [35]. They found that soaking and milling are significant factors in folic acid fortification; the optimum soaking time is 1.97 h; Folate retention rate follows 1<sup>st</sup> order kinetics; and the rates of natural rice hydrolysis (starch gelatinization) and folate uptake are both time-dependent. The initial experimental conditions used in this study of multi-micronutrient fortification of parboiled rice were taken from the optimum conditions found by Kam et al. [35]. In zinc fortification of whole grain rice using parboiling there is an increase in the retention of zinc about 1.3 to 4.5 times of the unfortified rice and more than half (64–100%) of the added zinc is retained after a simulated washing process before cooking [34]. In bioavailability studies of iron fortified parboiled rice increasing milling time and rinsing the iron-fortified parboiled rice decreases iron bioavailability [23]. The next step in the parboiling process, steaming, caused irreversible changes to the structure of the starch granules in the rice due to the application of moist heat (100°C). The kinetic energy of the starch increased and intermolecular hydrogen bonds were ruptured, leading to the disruption of structural integrity of starch [32] and starch gelatinisation. During starch gelatinization, the swollen starch may fuse with the inner bran and scutellum layers in the endosperm, preventing the loss of vitamin during further treatments such as milling [36].

### Loss of nutrients during parboiling in the soaking water

The uptake efficiency of the three micronutrients was not close to a



**Figure 3:** Retention of micronutrients in uncooked rice fortified by parboiling at varying concentrations and milling times (0 s, 60 s and 120 s). Error bars indicate the standard deviations between duplicates. (A, B, C and D refer to the concentration of the micronutrients added to the soaking water (Table 1) during parboiling at the optimized condition).

Nutrient loss	Combination	mg/300 g rice	Fresh*	Soaked*
β-carotene	β-carotene only	125	25.4 ± 7.3	0.4 ± 0.1
	β-carotene, folic acid	125,150	29.3 ± 16.4	0.6 ± 0.7
	β-carotene, iron	125,25	7 ± 3.6	1.1 ± 0.5
	β-carotene, folic acid, iron	125,150,25	23 ± 17.6	1.3 ± 0.6
Iron	iron only	25	1.3 ± 0.2	1.1 ± 0.2
	iron, β-carotene	25,125	1.1 ± 0.1	0.9 ± 0.1
	iron, folic acid	25,150	4.3 ± 2.9	0.8 ± 0.3
	iron, β-carotene, folic acid	25,125,150	1.9 ± 1.8	1.1 ± 0.3
Folic acid	folic acid only	150	0.6 ± 0.3	3.7 ± 0.4
	Folic acid, β-carotene	150,125	0.4 ± 0.1	3.0 ± 1.7
	Folic acid, iron	150,25	0.4 ± 0.2	3.9 ± 0.3
	Folic acid, β-carotene, iron	150,125,25	0.3 ± 0.2	2.2 ± 0.6

\*Mean ± Standard Deviation, unit expressed in %

**Table 3:** Micronutrient quantity concentration in 600 mL of soaking solution for rice before and after parboiling process (mg/300 g).

100%. This means that the nutrients are lost in the soaking water itself this is the loss of micronutrients during the parboiling process. Folic acid showed highest loss during parboiling in the soaking water (Table 3). Before parboiling, folic acid loss was 0.3-0.6% in soaking water but increased to 2.2-3.9% after parboiling. According to Kam et al. [35], water penetration into the rice grain at certain soaking durations was due to the availability of a constant amount of water. Rice fortified with folic acid and Fe contained the highest folic acid concentration in the soaking water, indicating low folic acid retention in the rice. Kam et al. [35] suggested that decreasing folic acid uptake may be due to diminishing moisture gradient with soaking time and therefore, the fortificant availability to rice was decreased.

When β-carotene was analysed in the soaking water after parboiling, the nutrient loss ranged from 0.4% to 1.3% (Table 3). The β-carotene concentration in fresh soaking water was higher, 7-29.3%. Perhaps the majority of β-carotene diffused into the rice during soaking water uptake. When the soaking solution had the 3 fortificants (β-carotene, folic acid and Fe), β-carotene content in the soaking water after parboiling had the highest loss (a high concentration of β-carotene remained in the soaking water). Similarly, Fe content in the soaking water also decreased after parboiling, compared to that fresh soaking water before the parboiling process. In fresh soaking water, Fe content was 1.1-4.3%, while it was approximately 1% in all samples after soaking (Table 3). In soaking water after parboiling with the 3 fortificants added, Fe had the lowest concentration, while the soaking water with iron and folic acid added had the highest Fe concentration. Nevertheless, the values of Fe concentration were similar, indicating high level of Fe retention in the grains after soaking.

However, the results were not statistically significant ( $p > 0.05$ ) when the analysis of variance was carried out. No correlation was found between β-carotene, Fe and folic acid concentration in fresh ( $p = 0.22; 0.15; 0.46$ , respectively) and soaked samples ( $p = 0.27; 0.62; 0.18$ , respectively) in the presence of other nutrients. With  $p > 0.05$ , it is therefore conclude that there are no mean differences between groups (i.e. fresh and soaked samples) in the population.

Overall, the uptake of nutrients by the rice grain during parboiling can be effective. However, there was still a certain magnitude of nutrient loss during the parboiling process from the soaking solution into the rice grains.

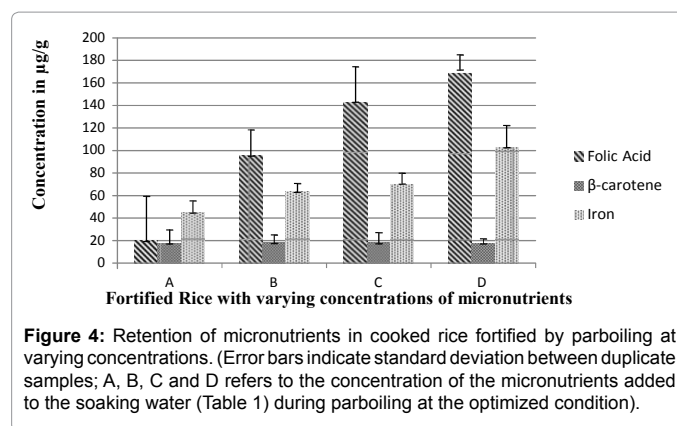
### Loss of nutrients on milling and cooking

During parboiling while soaking the rice with the nutrients,

penetration of the nutrients into the rice kernel occurred: some into the bran and some into the endosperm. Fortified rice was milled at 0 s (no milling), 60 s and 120 s and the retention analysis was done for folic acid, β-carotene and iron. With the increase in milling time there was a decrease in the retention of the micronutrients. Because folic acid was more concentrated on the outer surface of the grain [35], longer milling meant more folate loss.

Even though milling for 120 s resulted in 11% bran removal, the product seemed suitable for consumption when diluted with normal white rice in the ratio 1:10 with unfortified white basmati rice (by mixing overnight on a roller mixer). The nutrient retention after cooking by absorption for rice that was milled at 120 s was analysed (Figure 4). Nutrients were lost on cooking. Folic acid was well retained followed by iron. β-carotene which was the least retained.

The higher the concentration of fortificants added to the rice the less was the % retention. At higher concentrations there was higher loss of fortificants and the uptake was poorer (Tables 4 and 5).



**Figure 4:** Retention of micronutrients in cooked rice fortified by parboiling at varying concentrations. (Error bars indicate standard deviation between duplicate samples; A, B, C and D refers to the concentration of the micronutrients added to the soaking water (Table 1) during parboiling at the optimized condition).

Sample Name	Folic Acid	β-carotene	Iron
<sup>a</sup> A 0s	26	44	107
<sup>a</sup> A 60s	24	29	102
<sup>a</sup> A 120s	4	24	102
<sup>a</sup> B 0s	26	30	59
<sup>a</sup> B 60s	22	18	46
<sup>a</sup> B 120s	21	14	41
<sup>a</sup> C 0s	25	11	35
<sup>a</sup> C 60s	22	9	29
<sup>a</sup> C 120s	21	8	26
<sup>a</sup> D 0s	14	17	23
<sup>a</sup> D 60s	11	12	19
<sup>a</sup> D 120s	7	3	18

<sup>a</sup> denotes no significant difference between samples

**Table 4:** Percentage retention of micronutrients in uncooked rice at varying concentrations and milling time.

Varying concentrations of Fortificants	Folic Acid	β-carotene	Iron
Condition A	98	35	53
Condition B	46	30	92
Condition C	35	27	83
Condition D	65	39	84

**Table 5:** Percentage retention of micronutrients in rice (milled at 120s) after cooking at varying concentrations of the fortificant.

Therefore the lowest concentration of fortificant was better than high concentration although with higher concentrations of the fortificants the concentration for each micronutrient was higher after fortification. For iron soaked at the lowest concentration the uptake was close to a 100%, perhaps because the innate iron in the brown rice contributed to the final concentration. Since the retention of iron in the raw rice was over 58% this could be possible (data not shown).

Table 5 shows the % retention of micronutrients after cooking. For folic acid the highest retention was at the lowest concentration of fortificant mix after cooking the rice. Therefore this means that when low amount of fortificant is added the % retention is higher after cooking. This could minimize the amount of fortificant needed to be added when a food is being fortified by the principle of diffusion as long as the actual concentration was adequate. For  $\beta$ -carotene highest retention was at condition D followed by condition A and for iron, the highest retention was at condition B. It is evident that iron is more stable than the other micronutrients being a mineral and it is least affected by the cooking process in fortified rice compared to folic acid and  $\beta$ -carotene. In India, the practise of washing the rice before cooking is common. Due to this about 20 to 100% of the vitamins are lost depending on the amount of water used in rinsing and the length of cooking time [37]. In ultra-rice (extruded fortified rice) vitamin A loss was measured under normal rice cooking conditions (approximately 5 min boiling followed by 20-25 min under low-heat) and it was deduced that there was a loss of approximately 26% due to cooking [38]. Food folates are lost on cooking by 50%, however folic acid is more easily absorbed and less affected than folates upon cooking [39]. On the whole, lower concentrations of fortificants seemed effective in terms of uptake during parboiling and after cooking the fortified rice thus proving the robustness of the fortification process because three micronutrients could be fortified into rice by a single processing method.

Based on the Food and Nutrition Board (2010) (United States), 100 g of the fortified diluted rice intake per day would be able to satisfy the RDI for folic acid and  $\beta$ -carotene with the lowest concentration of the fortificants added during the parboiling process [40]. However for iron higher amounts are need to match the RDI at least double the given concentration [41]. Therefore, based on the prevalence of the deficiency among a given population, the concentration and combination of the micronutrients could be adapted to fortify the rice using the parboiling technique.

### Pilot scale studies of experimental condition in comparison to conventional parboiling condition

The retention of the nutrients after soaking for 2 hours and 4 hours is presented in Figure 5. There was little difference between the different soaking times for folic acid and iron, but for  $\beta$ -carotene the retention was higher during 2 hours soaking than for 4 hours soaking. In the study conducted by Kam, Arcot and Ward in 2012, it was found that progressive increment in the degree of gelatinization coincided with the increasing folic acid uptake for prolonged soaking durations (up to 4 hours). Hence it was deduced that starch gelatinization may be a potential contributor to retain or bind to the folic acid (fortificant) that had diffused into the endosperm from the soaking water.

There was no significant difference between the soaking times (2 hours and 4 hours) and different milling times using single factor ANOVA with  $p > 0.05$  ( $p = 0.7$  for folic acid;  $p = 0.3$  for  $\beta$ -carotene;  $p = 0.9$  for iron). Folic acid was retained more followed by  $\beta$ -carotene and then iron. Retention of nutrients for 0 s-120 s milling was 415-230 mg/kg for folic acid; 247-86 mg/kg  $\beta$ -carotene; 53-39 mg/kg for iron (Figure 5).

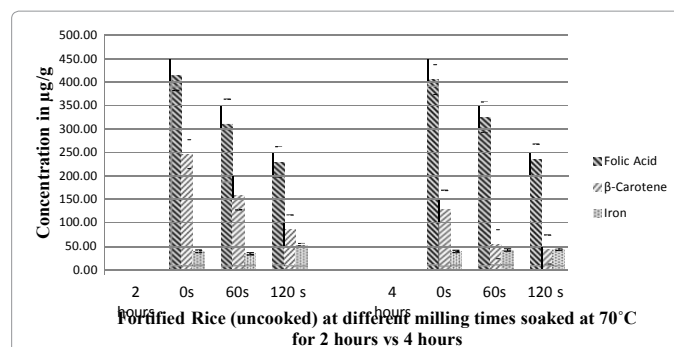


Figure 5: Comparison of micronutrient retention in uncooked rice using experimental condition (70°C for 2 hours) vs conventional condition (70°C for 4 hours) on pilot scale. Error bars indicate standard deviation.

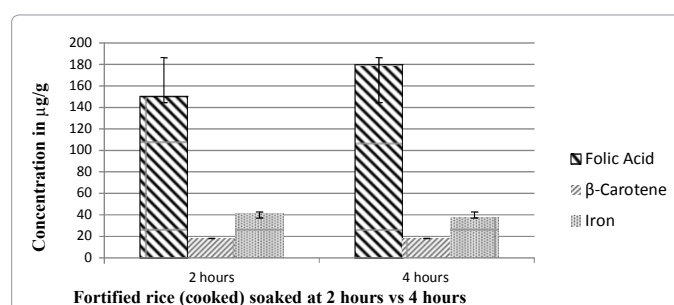


Figure 6: Comparison of micronutrient retention in cooked rice using experimental condition (70°C for 2 hours) vs conventional condition (70°C for 4 hours) on pilot scale. Error bars indicate standard deviation.

The retention of micronutrients in the scale-up study was similar to that of the lab scale study. There was no significant difference between the two methods (folic acid  $p = 0.3$ ;  $\beta$ -carotene  $p = 0.7$ ; iron  $p = 0.7$ ) using single factor ANOVA ( $p = 0.05$ ). However, slight variations between lab scale and pilot scale studies can be attributed to individual variation in the size and shape of rice kernels as the distribution of the nutrients varied with each rice kernel (Figure 5).

Statistical test showed that there was no significant difference between the two different soaking times even after cooking as shown in Figure 6 ( $p = 0.05$ ). Also the retention was quite similar to the lab scale fortification as well taking into account the standard deviation of the values.

### Conclusion

Fortification of rice with three micronutrients together using parboiling is feasible. The retention of micronutrients through this study was as follows: Folic Acid  $>$   $\beta$ -carotene  $>$  iron (at different milling times) in parboiled fortified uncooked rice with evident loss during milling. Overall when the rice was milled at 120 s, which was considered to be the optimum milling time in terms of consumer acceptance, it was found that approximately 12% of folic acid, and  $\beta$ -carotene and 46% of iron diffused into the uncooked rice during the fortification process. On an average about 60% of the folic acid, 33% of  $\beta$ -carotene and 78% of iron was retained after cooking the fortified rice. Retention of micronutrients on cooking was as follows: Folic acid  $>$  iron  $>$   $\beta$ -carotene. The fortified rice can be diluted with unfortified rice if used as a premix in the ratio 1:10. The retention after scale-up was similar to that of laboratory scale (single factor ANOVA). The efficiency of parboiling as a means of processing technique for rice fortification can be seen with the % uptake of the micronutrients in the uncooked rice and the

% retention of micronutrients in the cooked rice. Consumption of 100 g of the fortified rice diluted with white rice after cooking can provide at least half of RDI for folic acid and  $\beta$ -carotene. This could vary, depending on the country's rice eating pattern.

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