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Fermentation Reduces Anti-Nutritional Content and Increases Mineral Availability in *Poita bhat*

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Rice is a major source of dietary energy and nutrients for the people of Assam. However, due to the presence of antinutrional components like phytic acid, many of the nutrients, though present in the grain, is not readily bio-available for absorption by humans even after cooking. In the present investigation phytate and mineral contents of *Poita bhat*, a traditional fermented product was determined at different time interval of fermentation. The change in pH during fermentation was found to decrease with increase in time. The pH dropped from 6.80 to 5.39 within a 12 h period. The initial phytic acid content in the cooked rice (1.255 mg/g) decreased to 0.353 mg/g within the same time period. Whereas, the availability of the minerals like Fe, Mg, Ca and Zn increased with the passage of fermentation time. Magnesium content in *Poita bhat* was found to be highest followed by Ca. The Zn content was found to be the least among the all minerals analyzed. The phytate to mineral molar ratio were much lower than the critical values. Present study reveals that the traditional fermented rice is not only low in antinutritional factor but also a good source of minerals.

Keywords: *Poita bhat*, Mineral, Fermented Food, Phytate.

INTRODUCTION

Rice is the staple food grain of the Northeast India and the principal source of dietary energy and nutrition for the local populace. It is a good source of minerals like phosphorus, potassium and magnesium, dietary fiber and vitamins like B (thiamin, riboflavin and niacin) [1]. But, even after it is cooked, rice may not be completely digestible by the human body due to the presence of some antinutritional factor like phytate, trypsin inhibitor, oryzacystatin and haemagglutinin-lectin. Among these phytic acid or salts of phytic acid called as phytate [*myo*-inositol(1,2,3,4,5,6)*hexakis*phosphate] is recognized as one of the major antinutrient factor as it chelates positively charged metal ions such as calcium, magnesium, iron and zinc, making them bio-unavailable. In addition, phytic acid also binds to the positively charged proteins and forms insoluble protein aggregates. It is widely distributed at high concentrations in the plant feed stuff like rice and acts as a principal storage form of phosphorous [2-5]. Phosphorous is tightly bound to the phytic acid molecules and thus, remains unavailable to the human body. Moreover, phytate also inhibits the key digestive enzymes in human body including α-amylase [6,7], lipase [8], pepsin, trypsin and chymotrypsin [9-11].

Poita bhat is fermented rice that is partaken after fermenting the cooked rice for overnight. Usually leftover cooked rice is soaked in water and allowed to ferment overnight and consumed the next morning as a breakfast along with salt, chilly and lemon. The dish, slightly sour in taste with soft texture is considered to be a coolant during the summer and well regarded for its nutritional and therapeutic value. *Poita bhat* is also prepared to prevent the spoilage of leftover rice. The dish is also prepared by the rural populace of several Indian states and called as *Panta bhath* in Bengal, *Pokhalo* in Orissa, *Pazhankanji* or *Vellachoru* in Malayalam, *Pazhaya saadam* in Tamil and *Paaniwala chawal* in Hindi and popular in nearby Bangladesh where it is known as "*Pakhal*". In the present study we aimed to evaluate the nutritional properties of *Poita bhat* in terms of antinutrient content and mineral availability.

EXPERIMENTAL

Collection and preparation of sample: Rice sample (var. Ranjit) was purchased from the grocery shop and was used for preparation of *Poita bhat*. 100 g of rice of was cooked in pressure cooker and then cooled for 1 h in open tray. After that 250 mL of water was added and allowed to ferment for 12 h.

Changes in pH profile during fermentation: The pH of the sample was measured at an interval of 3 h using a digital pH meter (Type 361, Systronics, India).

Phytic acid content in *Poita bhat*: Estimation of phytic acid was carried out as per the method described by Sadasivam and Manickam [12]. The phytate of the sample was extracted with trichloroacetic acid and collected as precipitate of ferric salt. Iron content of the precipitate was determined colorimetrically by taking absorbance at 480 nm and the phytate phosphorus content was calculated from this value assuming a constant 4Fe:6P molecular ratio in the precipitate. Ferric nitrate [$Fe(NO_3)_3$] was used as the standard solution to measure iron in µg. The µg Fe present in the test sample was estimated from the standard curve and phytate phosphorus content was calculated as per the following equation:

$$\text{Phytate P (mg/g of sample)} = \frac{\text{µg of iron} \times 15}{\text{Weight of sample (g)}}$$

Bioavailability of minerals: Mineral analysis was carried out according to the methods described by Association of Official Analytical Chemists [13]. 1 g of sample was converted to ash in a muffle furnace (550 °C) followed by the boiling of the ash in 10 mL of 20 % hydrochloric acid. The solution was then filtered and the final volume was made up to 100 mL with deionized water. This solution was used to determine the mineral content. Potassium content was determined using the standard flame emission photometer using KCl as the standard [13]. Phosphorus was determined calorimetrically using the colorimeter 115 (Systronics, India) with KH_2PO_4 as the standard. Calcium, magnesium and iron were determined using atomic absorption spectrophotometer (Chemito, AA203D, India).

Molar ratio: Molar ratio of phytic acid:mineral was calculated by converting of phytic acid and minerals content from mg/g to mmol/g taking the molecular mass unit of 660.8 for phytic acid and atomic mass unit of each mineral respectively. Corresponding molar ratios were then calculated using these values.

RESULTS AND DISCUSSION

Changes in pH profile during fermentation: The pH of soaked rice decreased from 6.8 to about 5.39 after 12 h of fermentation. The decrease in pH during cereal fermentation was reported for different fermented foods [14,15]. The cause of drop in pH during cereal fermentation is due to the activity of lactic acid bacteria who utilize the free sugars to produce lactic acid [16-18]. The decrease in pH of the fermented food also enhances microbial enzyme activity that imparts additional benefits to the fermented foods. In addition, the low pH of fermented foods retards the growth of some other probable harmful microorganisms and helps in food preservation.

Phytic acid content in *Poita bhat*: The phytic acid content of initial cooked rice and during the 12 h period of fermentation was estimated at different time interval and represented in Table-1. The phytic acid content in the initial cooked rice (0 h) was 1.255 mg/g which reduced to 0.313 mg/g after 12 h of fermentation. The reduction in phytic acid content due to fermentation is thought to stem from the activity of microbial phytase enzyme during fermentation leading to phytate degradation [19]. Luo *et al.* [20] reported similar results in their study of indigenous fermentation of faba beans. Reduction in phytate content during fermentation of bread and other wheat products have been reported by Marfo *et al.* [21]. Mohite *et al.* [22] reported reduction in phytic acid due to fermentation in different types of cereals and pulses based foods. Decrease in phytate content was also found in *Ogwo*, a Nigerian fermented food using lactic acid bacteria and yeast as starter cultures [23].

TABLE-1
MEASUREMENT OF CHANGE IN pH AND PHYTIC ACID CONTENT OF *Poita bhat* AT DIFFERENT INTERVALS OF FERMENTATION

Time (h)	pH	Phytic acid content (mg/g)*
0	6.80 ± 0.008	1.255 ± 0.002
3	6.72 ± 0.016	0.807 ± 0.003
6	6.50 ± 0.026	0.653 ± 0.001
9	5.94 ± 0.026	0.353 ± 0.002
12	4.89 ± 0.012	0.313 ± 0.004

*Values are given as mean ± Standard deviation (n = 3).

Bioavailability of minerals: The mineral content of the sample was measured before and after fermentation. It was found that the minerals content increased with increasing time of fermentation as shown in Table-2. Among the minerals, Mg availability was highest whereas, Zn had the least availability.

Iron content of the cooked rice was 0.453 ± 0.008 mg/g and after 12 h of fermentation Fe content increased to 1.351 ± 0.012 mg/g. The higher Fe content after fermentation may be correlated to the decrease in phytic acid content due to fermentation and subsequent release of Fe. Mohite *et al.* [22] reported Fe content increase after fermentation in cereal based fermented foods like *Kurdai, Bidbe* and *Gulgula, Idli Dosa, etc*. In another study on mung bean flour, it was found that the Fe content is more in fermented flour than the unfermented flour [24]. Steve [25] reported that Fe content of wheat flour increased to 1.50 mg/100 g from 0.3 mg/100 g after fermentation. Other studies on cereals based food also showed that fermentation increases the bioavailability of iron [26].

Phosphorous content of rice before fermentation was 0.410 ± 0.008 mg/g which increased to 0.804 ± 0.004 mg/g after 12 h of fermentation. The increase in phosphorus content after fermentation may be due to the hydrolysis of phytic acid and subsequent release of phosphorus. Bhatia and Khetarpaul

TABLE-2
MEASUREMENT OF MINERALS CONTENT AT DIFFERENT INTERVALS OF FERMENTATION

Time interval (h)	Mineral content (mg/g)*					
	Fe	P	Mg	K	Ca	Zn
0	0.453 ± 0.008	0.410 ± 0.008	2.660 ± 0.032	0.511 ± 0.008	2.020 ± 0.014	0.0209 ± 0.00
3	0.516 ± 0.004	0.450 ± 0.004	3.333 ± 0.024	0.740 ± 0.010	2.511 ± 0.002	0.0238 ± 0.00
6	0.917 ± 0.002	0.516 ± 0.003	4.838 ± 0.014	1.236 ± 0.006	2.557 ± 0.006	0.0249 ± 0.00
9	0.953 ± 0.003	0.619 ± 0.004	4.955 ± 0.022	1.446 ± 0.016	2.616 ± 0.005	0.0269 ± 0.00
12	1.351 ± 0.012	0.804 ± 0.004	5.323 ± 0.020	1.851 ± 0.010	4.511 ± 0.0155	0.0328 ± 0.00

*Values are given as mean ± Standard deviation (n = 3).

[27] reported that phosphorus concentration of whole wheat bread amended with sprouted chickpea, increased up to 8.8 % and 10.1 % after fermentation at 35 and 40 °C, respectively. The increase in phosphorus content in their sample was credited to microbial action that degrades phytic acid and consequently releases the bound phosphorus. However, Afify *et al.* [28] reported that phosphorus content of sorghum significantly decreased after fermentation compared to the unprocessed sorghum.

Magnesium content before fermentation was 2.6 mg/g which after fermentation increased to about 5.2 mg/g. Magnesium content of different fermented cereals and pulse based foods such as *Idli, Dosa, Chikni papad etc.* were found to increase after fermentation [22]. The increased Mg content of the samples after fermentation may also be due to reduction in phytate content by microbial action. Another factor that might have also influenced the Mg availability is decrease in pH after fermentation. Grynspan and Cheryan [29] reported that phytate:Mg complex with a molar ratio of 6:1 becomes completely soluble at pH < 5 which is also applicable for phosphorus.

Potassium content of unfermented rice was found 0.51 mg/g which increased to 1.85 mg/g after 12h of fermentation. Potassium content of wheat flour increased to 186 mg/100 g after fermentation which was found to be 133 mg/100 g before fermentation [25]. Increase in K content in *ogwo*, a fermented sorghum-Irish potato gruel after fermentation with different LAB and yeast cultures has been reported [23].

Fermentation also increased calcium content of the sample from an initial content of 2.0 to 4.5 mg/g. Steve [25] reported similar findings in fermented wheat flour where the Ca content increased to 120 mg/100 g from 101 mg/100 g after fermentation. An increased Ca content was found in *Nilamadana,* a millet based food, fermented with fungi, compared to its unfermented counterpart [30].

Zinc content of the fermented sample increased marginally to 0.03 mg/g from an initial reading of 0.02 mg/g. Increase in Zn content after fermentation was reported by Steve [25] in fermented wheat flour. However, Onwurafor *et al.* [24] reported a decrease in Zn content after fermentation of Mung Bean flour and suggested that microbes involved in fermentation may utilize the Zn during their growth. A similar decrease in Zn content was also reported by Dwivedi *et al.* [30] in *Nilamadana,* a cereal based fermented food. Zinc content was also found to be lowest in fermented wheat flour compared to other minerals tested [25]. In this study among the minerals estimated, Zn content was found to be the lowest, which may be due to less Zn content in the rice sample tested.

This finding indicates that *Poita bhat* would serve as good sources minerals including iron, phosphorous, magnesium, potassium, calcium and zinc. Among these calcium and phosphorous are essential component of bone and teeth and deficiency of both lead to abnormal bones and teeth formation as well as retard development in children. Potassium plays important role in regulating nerve impulse and counter balance of sodium. A high intake of potassium has been reported to protect against increasing blood pressure and other cardiovascular risks [31]. Magnesium and zinc are components of enzymes and deficiency of these minerals may lead to adverse condition. Iron is the component of haemoglobin and deficiency of Fe leads to anemia is well documented.

Phytate:minerals molar ratio: The molar ratio of phytate:iron and phytate:calcium and phytate:zinc is shown in Table-3 along with their critical values. The critical values are the molar ratio of the phytate to minerals, greater than which lead to impair absorption of minerals. The phytate to mineral molar ratio describes the bioavailability of minerals. In this study, the phytate:iron molar ratio decreased from 0.23 before fermentation to 0.021 after fermentation. Phytate: calcium ratio of the rice samples ranges from 0.037 before fermentation which reduced to 0.004 after fermentation. The phytate:zinc molar ratio was 6.3 before fermentation which became 0.96 after fermentation.

TABLE-3
PHYTATE:MINERALS MOLAR RATIO

Time	Phytate:Fe	Phytate:Ca	Phytate:Zn
0 h	0.231	0.037	6.3
12 h	0.021	0.004	0.965
Critical values	> 1.0 [Ref. 33]	> 0.24 [Ref. 32]	> 15.0 [Ref. 34-36]

The phytate:minerals molar ratios of *Poita bhat* samples before and after fermentation were lower than the critical values indicating higher bioavalability. The lower molar ratio before fermentation may be due to the effect of milling and cooking that decrease the level of phytate. The ratio greater than the critical value (1.0, 0.24 and 15.0 for Fe, Ca and Zn respectively) have been associated with iron, calcium and zinc deficiency [32,37-40]. These minerals are regarded as essential trace elements for human nutrition [41] and deficiency of these elements have adverse effects on the growth development of children as they are most vulnerable to be deficient in these minerals [42]. The lower phytate to minerals molar ratio of *Poita bhat* indicates that the minerals present in this food are bioavailable.

Conclusion

Poita bhat is rice based traditional fermented food prepared in many house hold all over the Assam mostly during summer season. Our study shows that due to fermentation, the nutrient content of rice increased with simultaneous decreases in the antinutrient content. This traditional food can be better source of minerals and should be encouraged to prepare in house hold which may reduce food loss as well as mineral deficiency.

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