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In vitro iron bioavailability from value added traditional breakfast recipes incorporated with millet mix

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Abstract

Iron deficiency (ID) is one of the most common micronutrient deficiencies in the world. In addition to inadequate intake of iron, infection and blood loss are common causes of ID .Low cost intervention strategies that enhance the intake of dietary iron is a practical logical approach to combat iron deficiency especially among children . In this study, commonly used breakfast recipes in South India such as idli ,dosa , idiyappam, kichadi and kozhukattai were standardized and incorporated with millet mix powder comprising of barnyard millet , pearl millet, little millet and kodo millet . The breakfast recipes prepared with millet mix had significantly high iron content ($p<0.01$) compared to the standard recipe. In vitro bioavailability of iron was highest in dosa (46.93%) followed by kozhukattai (44.98%) idli (42.05%) kichadi (32.14%) and idiyappam (29.02%).Bioavailable iron was higher at lower pH, and as pH increases a decreasing trend on soluble and ionizable iron was observed. The study suggests that the promotion of consumption of millet incorporated foods can be an affordable simple strategy to increase iron intake. The findings reiterate the benefits of food based approaches for enhancing iron status of vulnerable segments of the population.

Keywords: Small millets, iron deficiency, breakfast recipes, dietary iron, in- vitro iron bioavailability

Introduction

Iron deficiency (ID) is one of the most common micronutrient deficiencies in the world. Anemia which is predominantly caused by ID is a major public health problem affecting nearly a third of the global population (Lopez et al., 2016). In India, it has been reported that around 0.5 per cent of total deaths in 2016 were contributed by nutritional deficiencies, particularly iron (Gonmei and Toteja,2018). There is substantial evidence indicating that iron deficiency is a consequence of decreased iron intake, inadequate absorption of iron, increased iron loss from the body or increased iron requirements. There is also growing evidence suggesting that impairment of mucosal absorption of iron may contribute to high rates of ID (Chandyoet al.,2016, Shah,2004) and that

iron absorption by the gut enterocytes controls iron balance (Dasa and Tilahun, 2018).

The degree of absorption of iron can vary dramatically depending on the type of dietary iron one consumes. There are two forms of dietary iron viz.,heme and non-heme iron. Heme iron is found in animal foods that originally contain hemoglobin, such as red meat, fish, and poultry. Iron in plant foods such as lentils and beans is arranged in a chemical structure called non-heme iron (Hurrell,1997). This is the form of iron added to iron-enriched and iron-fortified foods. Heme iron is absorbed better than non-heme iron, but most dietary iron is non-heme iron (Miret et al.,2003). Absorptionof heme from meat is efficient and ranges from 15-35 percent and is not significantly affected by the diet (Monsen,1988). In contrast, only 2 % to 20 % of non-heme iron in plant food such as maize, beans, soybeans and wheat is absorbed (Tapiero et. al., 2001) and is influenced by various food components (Dasa and Tilahun, 2018;Hurrell, 1997; Monsen, 1988).

It is well established that components in the diet like phytic acid which is found in cereal grains and legume seeds is the most potent inhibitor of iron absorption(Hallberget al.,1989). Also, tannins (found in tea), calcium and polyphenols and some proteins found in soya bean can decrease absorption of non-heme iron (Dasa and Tilahun, 2018;Lonnerdal, 2000). On the other hand, vitamin-C and meat enhance iron absorption.Non-heme iron is much more sensitive to these factors than heme iron, and depending on the composition of a meal, absorption can vary upto 20-fold (Dasa and Tilahun, 2018; Anbu Malar,2015). Thus adequate iron availability can be achieved principally by minimizing inhibitors (phytates, tannins) and enhancing promoters such as ascorbic acid and meat/fish (Nair and Iyengar,2009) Further, gastric pH is also found to have an impact on solubility of iron. Majority of dietary non-hemeiron is insoluble and thus inaccessible as it enters the GI tract in the ferric form and needs to be converted to the ferrous form for absorption. Acidic pH is essential and critical for iron to be in the soluble ferrous form which in turn determines its subsequent intestinal bio accessibility (Nair and Iyengar , 2009) Apart from these factors, Dasa and Tilahun (2018) reported that cooking techniques can also influence iron absorption. Soaking, germination and fermentation can comparatively reduce the phytate content of cereals thereby increasing iron absorption.

Understanding the significance of iron and its bioavailability in the body is very crucial since iron deficiency can lead to a plethora of detrimental consequences in the body. Many studies have proved that iron deficiency and anemia have adverse effects on growth and development, mental and neuromotor performance,immune-competence, physical working capacity, cognition, behavior and overall reproductive performance of affected individuals (Stoltzfus, 2003; Stoltzfus et. al., 2001 Grantham-McGregor and Ani, 2001; Haas and Brownlie, 2001 ;Scrimshaw, 2000; Gillespie and Johnston, 1998; Cook and Lynch, 1986). Studies have indicated that incorporation of millets into traditional recipes is beneficial in increasing micronutrient intake, in general, and iron in particular (Mounika et al.,2017;Anbu Malar, 2015). However, only few studies have examined the bioavailability of iron from millet based recipes. This is crucial because to translate physiological iron requirements into recommendations for dietary iron intakes, the bioavailability of iron (i.e., its absorption for utilization by the body) from different diets needs to be calculated. (Nair andIyengar, 2009). Hence this study was undertaken with the following objectives:

Objectives of the study

1. To develop and standardize selected millet mix incorporated breakfast recipes.
2. To assess the iron content of the breakfast items with and without millet mix.
3. To evaluate the bioavailability of iron from the millet mix incorporated breakfast items.

Materials and methods

Selection of Millets

For the present study, four millets viz., Pearl millet, Little Millet, Kodo Millet and Barnyard millet were chosen to enhance the nutritional content of traditional breakfast recipes. Pearl Millet (*Pennisetumglaucum*), is one of the four most important cereals (rice, maize, sorghum and millets) grown in tropical semi-arid regions of the world primarily in Africa and Asia. It has high energy, has less starch, high fiber (1.2g/100g, most of which is insoluble), 8-15 times greater α-amylase activity as compared to wheat, has low glycemic index (55) and is gluten free. (Bhat et al., 2018). According to Khairwal et al. (1997), pearl millet is rich in protein and iron (16.9mg/100g). Vanishaet.al., (2011), reported that pearl millet can be recommended in the treatment of celiac diseases, constipation and several non-communicable diseases. Little millet contains high phosphorous (220 mg/100g) and iron (9.3 mg/100g). Its complex carbohydrate digests slowly which is very helpful for diabetic patients. Among the small millets, kodo millet is a nutritious grain and a good substitute to rice and wheat (Bhatet.al., 2018). Kodo millet grain is composed of 8.3% protein and 2-3.5% iron. It is an excellent source of fiber (15%). Barnyard millet is a good source of protein (11.6%), which is highly digestible and it is also an excellent source of dietary fibre (13.5 g/100g) and iron15.2%. (Bhatet.al.,2018; Venna et al., 2005). Based on their nutrient benefits these four millets were chosen for the study,

Preparation Of Millet Mixes

The selected millets were purchased and cleaned (manually) thoroughly to remove dust, dirt, mud, clay and unwanted particles. Roasting was done at 60ºC for 15 minutes, in order to reduce the moisture content, improve flavour, and enhance shelf life and to destroy the anti-nutritional factors present in the millets (Makokha et al., 2002; Elyas et al., 2002 and SankaraRao and Deosthale, 1983).

Proportion Of Millet Used To Prepare Millet Mix For The Study

The proportion of millet mix (Barnyard millet- 40g/100g ; Pearl mill -40g/100g ; Little millet-10g /100g and Kodo Millet - 10g/100g) used in the study was based on the recommendation of an earlier study by Anbu Malar (2015). Gopalan et al., (2013) stated that the protein , iron and energy content of barnyard and pearl millet is much higher than that of kodo and little millet and hence these two millets were used in higher proportions.

Preparation of Millet Mix Incorporated Traditional Breakfast Items

Five common breakfast items such as idli, dosa, idiyappam , kichadi and kozhukattai were selected and standardized. Millet mix powder was added to the standard recipes in three varying proportions viz., 25%(Millet mix -MM1), 50% (Millet Mix ll –MM ll), and 75 % (Millet Mix lll –MM3). Organoleptic evaluation was done for different sensory attributes such as colour and appearance, flavour, texture, taste and overall acceptability with a maximum score of five (5 point Likert scale) for each of the food items prepared. The millet mix recipes which received the highest score were used for the study. The procedures for preparation of the breakfast items are given below:

Dosa

It is a crispy savoury pancake of South Indian cuisine. It is prepared by grinding rice and black gram dhal in a fixed proportion (3:1) which has been soaked for 6-8 hours, fermented and then made on a flat dosapan (Anbu Malar, 2015). For the present study 75% of dosa batter was replaced by millet mix flour batter.

Idli

It is a steamed recipe of South Indian cuisine. It is prepared by grinding rice and black gram dhal in a fixed proportion (3:1) which has been soaked for 6-8 hours, fermented and then poured into the idli plate which is placed in an idli cooker and steamed for 15-20 minutes . In the present study 25% of rice flour was replaced by millet mix flour.

Idiyappam

Idiyappam or string hoppers are a traditional recipe of Kerala and Tamil Nadu. It is made of rice flour or wheat flour, salt and water; kneaded into dough, extruded as strings and then steamed. It is generally served as the main course at breakfast or dinner together with coconut milk and sugar or gravy (Anbu Malar, 2015). For the present study, 25% of rice flour was replaced by millet mix flour.

Kichadi

Kichadiis a common South Indian breakfast dish, cooked as a thick porridge from dry roasted semolina i.e. white rava / wheat rava vermicelli. Various seasonings and/or vegetables are often added during cooking, depending on individual preferences. For the present study, 25% of semolina was replaced by millet mix flour.

Kolukattai

Kolukattaiis a popular South Indian sweet dumpling made from rice flour, grated coconut and jaggery and steamed. For the present study 25% of rice flour was replaced by millet mix flour

Iron Bio Availability Of Millet Based Recipes

Iron deficiency is one of the major nutrient deficiency problems among various segments of the population . In this scenario, millets are traditional crops of India and grow even in drought conditions. They are sources of carbohydrates, protein, fats, vitamins and minerals. It is a cheap crop and incorporation of millets into traditional recipes is one of the easiest ways to improve the nutritional status of malnourished children with all nutrients, especially iron in a single diet (Bhat et al., 2018).Therefore, the present study focused on iron bioavailability of millets.

Bioavailability is a measure which scores the absorbability of a nutrient by comparing it with that of a reference nutrient that is considered as having the most efficient absorbability.Anbu Malar (2015) summarized that bioavailability is the portion of the total ingested nutrient utilized by the body. Bioavailability is equated with absorption of a mineral element after its digestion from food and before its use in tissue and cells (Anderson et.al., 2004; Bilal,2002). According to Nair and Iyengar (2009) iron bioavailability is the absorption of iron for utilization by the body.

In the present study, in vitro bioavailability analysis were done for five foods incorporated will millet mix. Pepsin-HCL digestion procedure of Rao and Prabhavathi (1978) and Govindaraj, Krishna Rau and Prakash (2007) were adopted for assessing the invitro iron bioavailability. According to Rao and Prabhavathi (1978**)** the ionizable and soluble iron at pH 7.5 can be directly correlated with percent in-vivo iron absorption and the highest correlation with physiological availability in humans could be observed with ionizable iron at pH7.5. Based on this correlation the prediction

equation (Y= 0.4827 + 0.4707 X, where Y is the percent iron absorption in adult men and X is the percent ionizable iron at pH 7.5 for iron absorption using percent ionizable iron at pH 7.5) that was derived by the above authors was used in this study. The results were tabulated and statistically analyzed for further interpretation.

Statistical Analysis

Results of the study were statistically analyzed by SPSS 16.0 for mean and standard deviation. Students paired t -test was used to determine the significance of differences between the nutrient content of breakfast items with and without the incorporation of millet mix powder. Data were also analyzed using descriptive statistics.

Results and discussion

Table -1 clearly denotes that there was a significant increase in the iron content of millet mix incorporated recipes. This could be due to the fact that the iron content of millet mix was found to be 13.9±0.02mg/100g which was estimated using standard procedures for estimating iron content of foods. Rice contains (2.8%) protein and is the main ingredient in the preparation of idiyappam and kozhukattai as well as idli and dosa (NIN, 2017; Rao ,et al. 2017;Anbu Malar ,2015).The incorporation of 25% of millet mix to idli, idiyappam, kozhukattai and kichadi could be the reason for the significant increase in iron content of these millet mix incorporated breakfast recipes compared to the standard recipes. Millet mix dosa had the highest iron content (3.75±0.03,$p<0.001$) and this could be attributed to the fact that 75% of millet mix was incorporated into the standard recipe. With regard to kozhukattai, jaggery which is a good source of iron along with the incorporation of 25% of millet mix in the recipe could have contributed to the significant increase($p<0.01$)in its iron content (2.99±0.04mg/100g).

Table-2 represents the iron bioavailability of all the selected millet mix incorporated breakfast recipes. In the stomach, iron becomes soluble or ionizable when it comes in contact with HCL, whereas, the pH becomes alkaline when it enters the duodenum. On comparison with gastric pH, much of the iron is ionizable at acid pH and insoluble depending upon the factors in the diet (Prasad et al., 2014). Hence, the study estimated the soluble and ionizable iron at different levels. It is interesting to note that in the present study iron contents were higher at lower pH, and as pH increases a decreasing trend in soluble and ionizable iron was observed. This finding is accordance with Nair and Iyengar (2009) who reported the associationbetween gastric pH and solubility of iron and stated that acidic pH is essential and critical for iron to be in the soluble ferrous form which in turn determines its subsequent intestinal bioaccessibility.

Table -1 Iron Content of Breakfast Items Prepared With and Without Incorporation Of Millet Mix

Breakfast items	Standard recipe (mg/100g)	Millet mix incorporated recipe(mg/100g)
Idli	1.7±0.01	2.14±0.02**
Dosa	0.4±0.05	3.75±0.03***
Idiyappam	0.7±0.01	2.86±0.06**
Kichadi	1.7±0.02	2.8±0.04**
Kozhukattai	1.56±0.05	2.99±0.04**

Values are Mean±SD of three determinations. Data are significant differences at **$p<0.01$ and ***$p<0.001$

Table-2 In vitro Bioavailability of Iron From The Selected Millet Mix Incorporated Breakfast Items

S.No	Items	Total iron (mg)	Available iron (mg)	Available iron %	Soluble iron		Ionizable iron	
					1.35	7.5	1.35	7.5
1.	Kozhukattai	2.99	1.13	44.48	1.05	0.062	2.07	0.078
2.	Idiyappam	2.86	0.83	29.02	0.303	0.082	0.403	0.054
3.	Kichadi	2.8	0.9	32.14	0.471	0.109	0.401	0.094
4.	Dosa	3.75	1.76	46.93	0.491	0.350	0.519	0.382
5.	Idli	2.14	0.9	42.05	0.076	0.102	0.098	0.068

The total iron content of dosa was highest (3.75mg /100g) followed by kozhukattai (2.99mg/100g), idiyappam (2.86mg/100g) ,kichadi (2.8mg/100g) and idli(2.14.mg/100g) . However, the results clearly denote that percentage of iron bioavailability was highest in dosa(46.93%) followed by kozhukattai(44.48%), idli (42.05%) idiyappam (34.96%) and kichadi (29.64%). According to Nair and Iyengar(2009), the bioavailability of non-heme iron from commonly consumed plant based diets in India is estimated to be low due to an abundance of phytic acid and polyphenols.However,Mounika et. al., (2017) reported that food preparation techniques such as soaking, fermentation and cooking can reduce the levels of phytates , tannins exogenous and endogenous enzymes which can inhibit iron absorption. Cooking, in general, also increases the digestibility and absorption of iron which is improved by heating. During cooking, the food matrix softens and releases the iron (Dasa and Tilahun 2018;Mounika et al. 2017).

The bioavailability of iron from the selected millet incorporated breakfast items as observed in Table -2 could be attributed to the cooking process used for the study. The high bioavailability of iron from dosa and idli, in particular, could be due to the fact that both these recipes include 6-8 hours of soaking and fermentation of the cereals before cooking. This finding is in accordance with Dasa and Tilahun (2018) who reported that soaking, fermentation and cookingcan reduce the phytate content of cereals and eliminate their inhibitory effects on iron absorption. The findings reveal that the bio availability of iron from millet incorporated idli and dosa was higher than that of kichadi and idiyappam . This could be due to the fact that idli and dosa are prepared using a combination of cereal and pulse. Nair and Iynegar (2009) reported that a combination of cereals and pulses enhances iron absorption and this could be the reason for the finding. This study clearly highlights the bioavailability of iron from selected millet mix incorporated breakfast recipes. These results suggest that the incorporation of millets into traditional recipes is a simple cost effect technique to ensure iron bioavailability and subsequently improve the iron status of individuals. This in turn can help reduce the risk of iron deficiency anemia among them.

Conclusions and recommendations

Millet mix incorporated breakfast items such as idli, dosa, idiyappam, kichadi and kozhukattai had significantly higher content of iron than the breakfast items prepared using standard recipes. Iron bioavailability was highest in dosa followed by kozhukattai, idli, idiyappam and kichadi .The findings substantiate the benefits of small millets such as barnyard millet, pearl millet , little millet and kodu millet in

increasing iron intake. This, in turn, could help in alleviating iron deficiency. Awareness about the benefits of incorporating millets in traditional diets should be created among home makers and the general public. Since iron deficiency is a widespread problem, it is essential to promote the formulation of a wide variety of millet incorporated foods and popularize their usage.

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