



## QUALITY EVALUATION AND FORTIFICATION OF IRON RICH COOKIES USING MILLETS

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### ABSTRACT

Iron is essential to all body cells. Iron functions primarily as a carrier of oxygen in the body, both as a part of hemoglobin in the blood and of myoglobin in the muscles. Iron deficiency anemia occurs when there is not enough iron in the red blood cells. This is a common problem often caused by pregnancy, blood loss and a diet low in iron or poor absorption of iron by the body. Now, Iron deficiency anemia is a major public health problem affecting numerable people throughout the globe. There is substantial evidence that anemia in children is associated with decreased physical and mental development, impaired immune function and reduced capacity of leucocytes to kill microorganism. To overcome this anemia some traditional foods like millets are prescribed nowadays. Millets have been neglected despite their nutritive value and therapeutic use. Millets is a nutria-cereal which can be exploited for the use as functional foods. Millets grains are nutritionally comparable and superior to major cereals with respect to protein, energy, vitamins and minerals. They are rich source of dietary fiber, phytochemical and micronutrients. Hence the present study was carried out with the objective to prepare iron enriched cookies by supplementing millets flour to assess the nutritional quality.

**KEYWORDS:** Iron, Anemia, Millets, Cookies.

### INTRODUCTION

Iron is the fourth most abundant element on earth, but iron deficiency in humans is one of the most widespread nutritional problems in the world.<sup>[1]</sup> And it is a trace element which is needed by the body for the formation of blood. The human body normally contains 3-4g of iron, more than half of which is in the form of haemoglobin, the red pigment in blood. Haemoglobin transports oxygen from the lungs to the tissues. Iron is a constituent of a number of enzymes.<sup>[2]</sup>

Iron deficiency is the most common nutrient deficiency internationally. It occurs most often in young children, pregnant women, and menstruating women who lose iron through their monthly periods. Iron status is easily assessed with blood tests. Low iron levels can result in anemia. Symptoms of iron-deficiency anemia are fatigue, a fast heartbeat, and shortness of breath during physical activity.<sup>[1]</sup>

Anemia (hemoglobin level < 11 g/dL) remains one of the most intractable public health problems in malaria-endemic countries of Africa. Iron is critical for the growth of all cells. Not surprisingly, therefore, iron deficiency anemia independently increases morbidity and mortality.<sup>[3]</sup> There are an estimated 3.5 billion iron

deficient people worldwide, the vast majority in developing.<sup>[4]</sup> Remarkably, iron deficiency is also the most common cause of anemia in the United States.

Some anaemic women use herbal medicine and follow a suitable diet. Increased use of plants and natural products has been reported<sup>[5]</sup> and is linked to increased incidence of some chronic diseases, increased frequency of adverse drug synthesis and cost very high for some pharmaceutical products.<sup>[6]</sup> Today, herbal medicine is based on both tradition and scientific research according to official standards of modern medicine. In parallel with the use of plants in the treatment of some diseases, diet plays an important role in the prevention and mitigation of some chronic diseases.

Millets are major food sources for millions of people, especially those who live in hot and humid areas of the world. They are grown mostly in marginal areas under agricultural conditions in which major cereals fail to give substantial yields.<sup>[7]</sup> Millets, which are high in iron and cost-effective, should be encouraged for consumption in India and eventually aim at negating Iron Deficiency Anemia. Millets are claimed to be the oldest grown grain. Known as 'Gods own crops' 'Miracle Grains'/'Adbhut Anaj' 'Nutri Cereals' these grains owe it to the

nutrient richness they possess. Currently, India is the largest producer of a variety of Millets. It still is a staple crop. The millets cultivated in India are- Finger Millet, Foxtail Millet, Kodo Millet, Little Millet, Proso Millet, Barnyard Millet, Pearl Millet and Sorghum.<sup>[8]</sup> Hence, the objective of this research was to overcome the problem of iron deficiency anaemia by preparing iron riched cookies using millet flours.

## MATERIALS AND METHODS

### COLLECTION OF MILLET: (Anju *et al.*, 2010)<sup>[9]</sup>

Barnyard millet, Finger millet, Foxtail millet, Kodomillet, Little millet, Pearl millet, Proso millet and Sorghum grains were purchased from local farmers. The grains were cleaned free from dust and foreign particles and were subjected to grinding in a commercial roller mill. Thereafter it was passed through a 20 mesh sieve in order to obtain the flour for the study.

### ESTIMATION OF IRON

#### Qualitative method to determine iron (Spot test): (AACC method 40-40)<sup>[10]</sup>

Ferric iron, in an acidic medium, reacts with a solution of potassium thiocyanate (KSCN) to form an insoluble red pigment. Other types of iron, such as ferrous iron and elemental iron can also react in a similar manner once they are oxidized to the ferric form using hydrogen peroxide. In spot test, filter paper is placed over the watch glass and solution of potassium thiocyanate is sprayed to penetrate the surface of filter paper. Using hand sieve, sift portion of the flour sample in order to load a thin layer over the entire wet area and scrape off excess flour. Add a little more of the acidic solution of potassium thiocyanate over the flour layer and add small amount of the H<sub>2</sub>O<sub>2</sub> solution and leave for a few minutes for the reaction to occur (oxidation of any form of iron to iron(III)). Red spots indicate the presence of added iron from any source. Number and density of spots might be associated to the iron level in the sample.

#### Quantitative spectrophotometric method for determining iron (Phenanthroline method): (Hana Ali & Omar Dary, 1997)<sup>[11]</sup>

100g of flour were weighted and mix thoroughly. Take 10g of flour from the mixer with significance in milligram and pour slowly into 250ml Erlenmeyer flask, already containing 1.0g TCA dissolved in about 100ml of water: acetone (80:20). Stir it with a magnetic stirrer for 10 minutes. Seal the flask with parafilm and leave it in the refrigerator for at least 1 – 1.5 hr. Decant the supernant in equal amounts into two centrifuge tubes and centrifuged (3500rpm) for at least 15 minutes. Transfer the supernants and make up to 100ml with deionized water. Pipetted out 10.0ml of the sample solution and add 5.0ml acetate buffer and 4.0ml 1,10-phenanthroline to each flask and allow to stand for 30 minutes. The sample was read at 510nm in spectrophotometer. Standard graph was drawn plotting the concentration of Iron on X axis and optical density on Y axis. From the graph the amount of Iron ( $\mu\text{g/ml}$ ) presented in the sample

was calculated. **Estimation of Calcium and Magnesium: (Manivasam, 1995)**

### QUANTITATIVE ESTIMATION

#### Estimation of Protein (Lowry *et al.*, 1951)<sup>[12]</sup>

#### Estimation of Carbohydrates (Hedge and Hofreiter, 1962)

Carbohydrates are first hydrolyzed into simple sugars using diluted hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This compound reacts with anthrone and forms a green colored product with an absorption maximum at 630nm.

### Procedure

1.0 g of sample was taken into boiling tubes and hydrolyzed by keeping them in boiling water bath for 30minutes to 1 hr with 5ml of 2.5 N HCL. It was cooled to room temperature and neutralized with solid sodium carbonate until the effervescence ceases and made up the volume to 100ml and centrifuged. 0.5ml and 1ml of the supernatant were collected and used for further analysis. The volume was make upto 1.0ml in all the tubes including the sample tubes by the addition of distilled water. 4ml of 0.2% Anthrone reagent was added to each test tube followed by heated in a boiling water bath for 10minutes. Test tubes were cooled at room temperature. Dark green color was appeared on heating the samples. The optical density (OD) value of the colored solution was then measured through 630nm wavelength in a colorimeter against blank. Standard graph was drawn by plotting the concentration of Glucose on X axis and optical density on Y axis. From the graph the amount of carbohydrate (mg/ml) present in the sample was calculated.

### PROXIMATE ANALYSIS

#### Estimation of Ash (A.O.A.C, 1990)

#### Estimation of Moisture (A.O.A.C, 1995)

#### SYNERGISM OF THE FLOUR: (Islam *et al.*, 2012)

Of all the millets, only four millets which were rich in iron was selected for synergism. The millets which were choosen for synergism was Kodomillet, Foxtail millet, Pearlmillet and Fingermillets. The flour of these millets were subjected to various type of test including Iron, Calcium, Magnesium, Protein and CHO etc in different combination. From the result of synergism, Iron rich combination of the flour was selected and made the Biscuit formulation.

#### FORMULATION OF BISCUIT: (Anju *et al.*, 2010)<sup>[9]</sup>

Four types of biscuit namely Kodomillet biscuit (KMB), Foxtail millet Biscuit (FMB), Pearl millet Biscuit (PMB) and Fingermillet Biscuit (FIMB) were prepared. For all the biscuit, the flour blend was taken in different ratio. The other ingrediants used were similar for all three types of biscuits: brown sugar (30%) and unsalted butter (30%). All the ingrediants were creamed together and was prepared to the form of dough. The prepared dough was rolled into balls, pressed to a thickness of 1cm and 5cm diameter, and baked under standardized conditions.

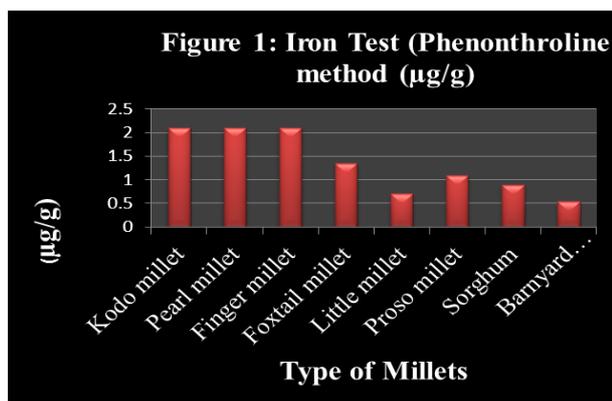
## RESULT AND DISCUSSION

The nutrient composition of different types of millet flour used for cookie preparation are shown in Table 1.

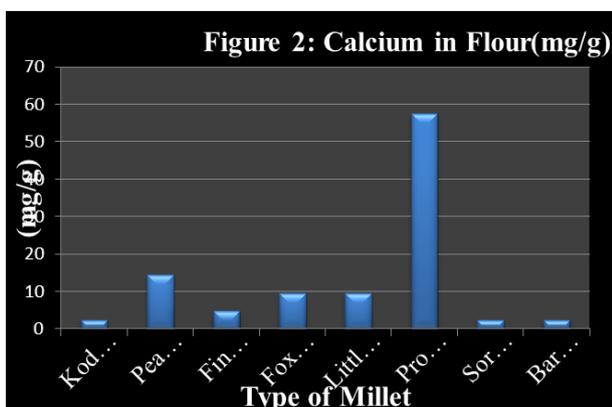
**Table 1: Nutrient composition of the flour.**

Types of Millets	Parameter				
	Iron test		Calcium (mg/g)	Magnesium (mg/g)	Carbohydrate (mg/g)
	Spot test	Phenanthroline test ( $\mu\text{g/g}$ )			
Kodo millet	Absent	2.10	2.40	4.00	55.00
Pearl millet	Present	2.10	14.40	24.00	53.00
Finger millet	Absent	2.10	4.80	8.00	62.92
Foxtail millet	Absent	1.35	9.60	4.30	62.86
Little millet	Present	0.70	9.60	4.30	62.70
Proso millet	Present	1.10	57.60	96.00	62.91
Sorghum	Present	0.90	2.40	4.00	62.64
Barnyard millet	Present	0.55	2.40	4.00	62.74

Iron content (spot test) were found to be higher in Pearl millet, Little millet, Proso millet, Sorghum and Barnyard millet while compared with kodo millet, Finger millet and Foxtail millet. Kodo millet, Pearl millet and Finger millet showed high iron content compared with little millet which is low in Iron.

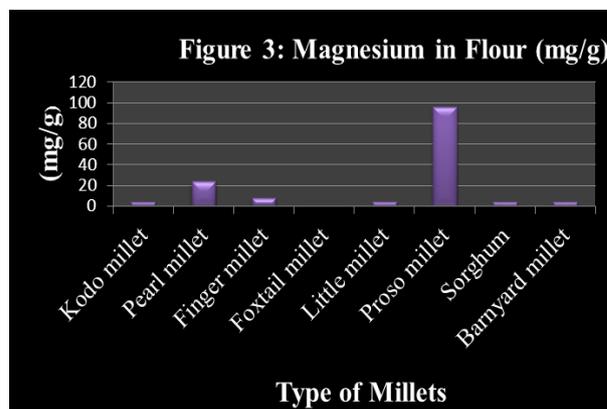


Calcium and Magnesium content were increased in Proso millet and decreased in Kodo millet, Sorghum, Barnyard millet, Foxtail millet and little millet.

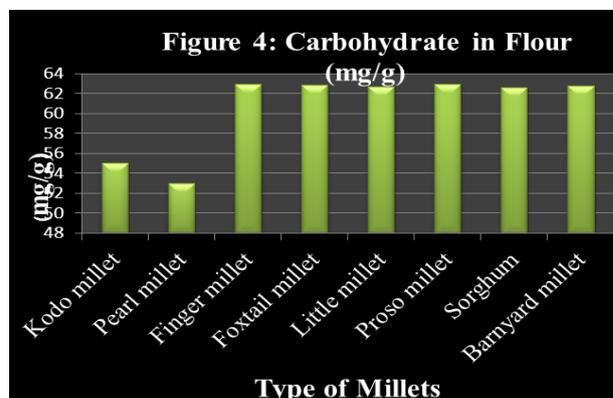


Increased level of Carbohydrate ranges (62.92%) in Finger millet and decreased level of (53%) in Pearl millet. Adekunle *et al.*, 2013<sup>[7]</sup> stats that the iron content in whole grains of minor millets varied from 44.92 ppm in Kodo millet to 46.20 ppm in Barnyard millets. The

observed values are higher than the average iron content present in major cereal crops such as rice, wheat, maize, etc. as well as other millets like sorghum and pearl millet.



Ferric iron, in an acidic medium, reacts with a solution of potassium thiocyanate to form an insoluble red pigment. Other types of iron, such as ferrous iron and elemental iron can also react in a similar manner once they are oxidized to the ferric form using hydrogen peroxide. Number and density of spots might be associated to the iron level in the sample. The more red spots appear, the higher the concentration of iron in the sample.



Sharma J 2007 resulted that the Finger millet has thirty times more Calcium than rice while every other millet

has at least twice the amount of Calcium compared to rice.

Table 2 showed that the synergism of millets in different ratio such as Kodo millet: Foxtail millet: Pearl millet: Finger millet (10:10:10:30).

**Table 2: Iron test for combination of the flour.**

S. No.	Combination of millet (K+F+P+FI) (g)	Iron Phenanthroline test ( $\mu\text{g/g}$ )
1.	7.5:7.5:7.5:7.5	7.00
2.	10:10:10:30	11.90
3.	10:20:20:20	19.90
4.	5:5:10:40	12.00
5.	15:15:10:20	16.00
6.	10:30:10:10	19.90
7.	10:10:30:10	1.00
8.	10:10:10:30	39.00

Where K= Kodomillet, F= Foxtail millet, P= Pearl millet, FI= Finger millet

The higher content of iron were obtained in those millets. The lower iron content were observed on (10:10:30:10).

Brasil, 1998 displayed the information, the final product must contain atleast 15% (2.1mg) of the DRV for each 100g, and should be designed 'rich in iron' it should contain at least 30% (4.2mg) of the DVR. Notwithstanding, both almond and peanut cookie could be considered 'source of iron' taking into consideration the Brazilian DRV for adults (14mg) and presented higher concentration of iron than the commercial cookie prepared with Brazilian nuts. Table 3 demonstrated the Basic formulation of Biscuits.

**Table 3: Basic formulation of Biscuits.**

S. No.	Ingredients	Biscuit Formulation (g)			
		A	B	C	D
1.	Kodomillet	10	30	10	10
2.	Foxtailmillet	10	10	30	10
3.	Pearlmillet	10	10	10	30
4.	Finger millet	30	10	10	10
5.	Brownsugar	30	30	30	30
6.	Unsalted butter	30	30	30	30

Where, A= Finger millet Biscuit, B= Kodomillet Biscuit, C= Foxtail millet Biscuit, D= Pearlmillet Biscuit

There are four different formulation of Biscuit namely Kodo millet biscuit, Foxtail millet biscuit, Pearl millet biscuit and Finger millet. The other ingredients for this formulation includes brown sugar and unsalted butter. Basic formulation of biscuit paid a way for further proceeding of the study.

Islam *et al.*, 2012 demonstrated the different formulation of cookie and analysed the physicochemical and functional properties of the cookies. Chemical composition of Finger millet biscuit is shown in Table 4.

**Table 4: Chemical composition of Finger millet Biscuit.**

S. No.	Name of Tests	Results
1.	Biscuit Weight (g)	10.15
2.	Iron ( $\mu\text{g/g}$ )	72.00
3.	Carbohydrate (mg/g)	83.00
4.	Calcium (mg/g)	30.00
5.	Magnesium (mg/g)	70.00
6.	Protein (mg/g)	149.00
7.	Moisture (%)	91.00
8.	Ash (g)	0.04
9.	Taste	+++

Indicates“+++” = Good

The iron content of Finger millet biscuit is calculated as 72.00 $\mu\text{g/ml}$ . Protein, Calcium, Magnesium and Carbohydrates content were found to be 1.49mg/ml, 0.30mg/ml, 0.70mg/ml and 0.83mg/ml.

Pragy Singh *et al.*, 2012 resulted that SINM had moisture, crude protein and total ash content of 8.83,23.68 and 4.51 respectively. Carbohydrate content was 54.9% and Iron and Calcium were 23.80 and 517.37mg/ml while ionisable iron was 878  $\mu\text{g/ml}$ . The value of in vitro protein digestibility was 62.34% of crude protein while in vitro iron bioavailability was 28.67%.

Pore *et al.*, 1979 demonstrated that the finger millet is good source of nutrients especially of calcium, other minerals and fibre. Total carbohydrate content of finger millet has been reported to be in the range of 72 to 79.5%. Chemical composition of Kodo millet biscuit is shown in Table 5.

**Table 5: Chemical composition of Kodomillet Biscuit.**

S.No	Name of Tests	Results
1.	Biscuit Weight (g)	10.0
2.	Iron ( $\mu\text{g/g}$ )	2.50
3.	Carbohydrate (mg/g)	124.00
4.	Calcium (mg/g)	40.00
5.	Magnesium (mg/g)	50.00
6.	Protein (mg/g)	1500.00
7.	Moisture (%)	19.20
8.	Ash (g)	0.07
9.	Taste	++

Indicates“++” = good

Protein, Calcium, Magnesium and Carbohydrates content were found to be 1500.00mg/ml, 0.40mg/ml, 0.50mg/ml and 124.00mg/ml. The iron content of Finger millet biscuit was resulted as 2.50 $\mu\text{g/ml}$ . Chemical composition of Pearl millet biscuit is shown in Table 6.

**Table 6: Chemical composition of Pearl millet Biscuit.**

S.No	Name of Tests	Results
1.	Biscuit Weight (g)	11.85
2.	Iron ( $\mu\text{g/g}$ )	25.00
3.	Carbohydrate (mg/g)	70.00
4.	Calcium (mg/g)	60.00
5.	Magnesium (mg/g)	90.00
6.	Protein (mg/g)	660.00
7.	Moisture (%)	19.10
8.	Ash (g)	0.09
9.	Taste	+++

Indicates“+++” = Better

The iron content of Finger millet biscuit is calculated as 25.00 $\mu\text{g/ml}$ . Protein, Calcium, Magnesium and Carbohydrates content were measured to be 660.00mg/ml, 0.60mg/ml, 0.90mg/ml and 70.00mg/ml.

The moisture content ranged between 3.0 and 4.1% with minimum moisture content in pearl millet: sorghum combination of cookies (Table 2). Protein content ranged from 5.8% (rice: maize) to 7.4% (pearl millet: sorghum). The pearl millet: sorghum cookies had highest fat (19.2%), protein (7.4%), ash (1.6%) and calorific value (481.7 Kcal/100 g) with minimum moisture content and found to be best for nutritive value. Similarly, Rajput *et al.* (1988) reported that biscuits containing unconventional protein sources increased the protein content in biscuits from 5.9 to 11.5– 18.1%. Semwat *et al.* (1996) reported that maximum variation was found in moisture (2.47–8.75%) and fat content (1.04–14.82%) in different commercially available biscuits.

Vanisha *et al.*, 2011<sup>[13]</sup> revealed that the carbohydrate content of pearl millet is 67.5g/100g, which is lower than wheat, rice and sorghum, but higher than maize (Table 1) as per the Nutritive value of Indian foods (NIN, 2003). The protein content of pearl millet is comparable to wheat (11.6 vs 11.8 g/100g), is higher than rice (6.8 g/100g), sorghum (10.4 g/100g) and maize (4.7 g/100g) as per the Nutritive value of Indian foods (NIN, 2003). Chemical composition of Foxtail millet biscuit is shown in Table 7.

**Table 7: Chemical composition of Foxtail millet Biscuit.**

S. No	Name of Tests	Results
1.	Biscuit Weight (g)	10.85
2.	Iron ( $\mu\text{g/g}$ )	32.50
3.	Carbohydrate (mg/g)	62.00
4.	Calcium (mg/g)	100.00
5.	Magnesium (mg/g)	180.00
6.	Protein (mg/g)	680.00
7.	Moisture (%)	20.00
8.	Ash (g)	0.05
9.	Taste	+++

Indicates“+++” = Better

Iron content of Finger millet biscuit was found to be 32.50 $\mu\text{g/ml}$ . Protein, Calcium, Magnesium and Carbohydrates content were found to be 680.00mg/ml, 1.00mg/ml, 1.80mg/ml and 62.00mg/ml.

Anju *et al.*, 2010<sup>[9]</sup> reported that the value for crude protein, total ash, carbohydrate and Magnesium content of foxtail millet ranges from 5.88%, 1.1%, 52.77% and 33.9 respectively.

## CONCLUSION

Different types of millets had been taken for my study and its iron content were determined by various methods. High iron content were seen in Kodo millet (2.10 $\mu\text{g/g}$ ), Pearl millet (2.10 $\mu\text{g/g}$ ), Finger millet (2.10 $\mu\text{g/g}$ ) and Foxtail millet (1.35  $\mu\text{g/g}$ ) respectively while compared with other millets. Calcium, Magnesium and Carbohydrate were also observed. The synergism of millets in different ratio such as Kodo millet: Foxtail millet: Pearl millet: Finger millet were calculated and the ratio (10:10:10:30) showed higher iron content and the ratio (10:10:30:10) and the lower iron content were observed on (10:10:30:10).

Four different formulation of Biscuit were made using Kodo millet, Pearl millet, Finger millet and Foxtail millet based on their iron content. Kodo millet Biscuit, Pearl millet Biscuit, Finger millet Biscuit and Foxtail millet Biscuit contain iron in the range of 2.50 $\mu\text{g/g}$ , 25.00 $\mu\text{g/g}$ , 72.00 $\mu\text{g/g}$ , 32.50 $\mu\text{g/g}$  respectively. Calcium, Magnesium, Protein, Carbohydrate, Moisture and Ash were also Observed. Finger millet Biscuit (72.00 $\mu\text{g/g}$ ) showed high iron content while compared with other formulation.

From this study, I conclude that finger millet which had 60% of overall iron content can be successfully incorporated to formulate cookies. These cookies are low cost, nutritious and were acceptable at both laboratory and field level. So, it can be recommended to anemic patient instead of allopathic medicine which gives high degree of harmfulness and side effects. These types of products could increase in the market, offering new nourishing options of consumption for Iron intolerants.

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