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SPECTROSCOPIC ANALYSIS OF IRON CONTENT IN INDIGENOUS FRESH AND DRY BEAN SPECIES FROM KARACHI CITY

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ABSTRACT

Iron deficiency anaemia has a significant prevalence among Pakistani population, particularly children and women. The current study is aimed to establish the role of indigenous fresh beans as a significant source of iron, provide better selection of bean and track some factors that can vary ion content in beans. Purposely, five fresh beans, namely peas (*Pisum sativum*), chick peas (*Cicer arietinum*), hyacinth beans (*Lablab purpureus*), cluster beans (*Cyanosis tetragonoloba*) and French beans (*Phaseolus vulgaris*) were analysed spectrophotometrically using 1,10-phenathroline method for iron content. Dry chick peas and kidney beans (white and red) of different sizes were also analysed to establish a comparative account of iron content based on size, color and state (fresh/dry) in different beans. A significant difference (P<0.000) was noticed in the average iron content of different fresh bean samples. Among the selected fresh beans, cluster beans showed the highest mean iron content (10.9 ± 1.8 mg/100g) while the green chick peas showed the lowest average iron (4.6 ± 0.7 mg/100mg). The amount of iron in fresh beans was found significantly higher (P<0.000) compared to that in dry beans. Significant difference was observed among different sized dry beans. The amount of iron was found higher in small sized dry chick peas than large dry chick peas (P = 0.016). The iron content in small kidney beans was consistently higher than iron found in large kidney beans. The white kidney beans contained relatively higher iron content compared to that for red kidney beans. Interestingly, the samples collected from the polluted areas and the areas situated near the petrol pumps showed less amount of iron probably because of heavy lead contamination in the environment that reduces the iron absorption.

Key-words: *Pisum sativum*, *Cicer arietinum*, *Lablab purpureus*, *Cyamopsis tetragonoloba*, *Phaseolus vulgaris*, 1,10-Orthophenathroline, Fe-OPT Complex, Iron Deficiency Anaemia

INTRODUCTION

Iron is an essential mineral required for many biochemical functions in the human body, microorganisms, plants and animals. Iron is associated with the formation of hemoglobin, regulation of body temperature, development and functioning of brain, catecholamine metabolism and muscle activity. The role of iron is central in cellular respiration and oxygen transport (Sarkar *et al.,* 2018). The adult human body requires 14 mg of iron per day (Carvalho *et al.*, 2012). Lower iron levels may result in iron deficiency anaemia (Baumgartner and Barth-Jaeggi, 2015). The iron deficiency is a most common and widespread nutritional disorder in the world, particularly prevalent in low-income developing countries among children and pregnant women (Pereira *et al.*, 2014; Martins *et al.*, 2016).

Globally, iron deficiency anaemia (IDA) is the leading cause of problems relevant to malnutrition. This may result in a number of pathological complications such as immunosuppression, stunted growth, pregnancy complications, impaired cognitive and motor development in children with increased morbidity and mortality risk, etc. (Ahmed *et al.,* 2014; Danquah *et al.,* 2014; WHO, 2017; Dignass *et al.,* 2018). The leading cause of this deficiency is the inadequate intake of the iron rich foods or the unavailability of iron in the foods (Carvalho *et al.*, 2012; McDonagh *et al.,* 2015). IDA is mainly due to low meat consumption or significant dietary reliance on grains enriched with iron absorption inhibitors, such as phytic acid and polyphenolic compounds (Tako *et al.,* 2015).

Consumption of legumes as a food has its roots throughout the history of mankind. The common bean (*Phaseolus vulgaris*) is the major staple food in various regions and is one of the most important legumes worldwide (Petry *et al.,* 2012). Cowpea (*Vigna unguiculata*) is another significant food legume in the developing countries of Asia, Latin America, and Africa, available at a low cost (Pereira *et al.,* 2014). The food beans are served as the potential source of vegetable proteins, fibers, vitamins and minerals (e.g., Fe, Zn, Mg, Cu, Mn, Ca and P), and thus they play a significant role in regulating the enzymatic metabolism (Nchimbi-Msolla and Tryphone, 2010; Huang *et al.*, 2014; Maurya *et al.,* 2015). Pakistan, being an agricultural country, is rich in these food resources. For example, cluster bean (*Cyamopsis tetragonoloba* or *C. psoraloides*), also called Guar, is being cultivated as a food crop in

Indo-Pakistan subcontinent for centuries with most concentrated (about 80%) world growth of guar seed distributed in these regions (Mudgil *et al.*, 2011). However, the data on the quantitative analysis of the constituents of these indigenous beans is scarce. Furthermore, the populations of South and Southeast Asia are among the regions that are at the highest risk of iron and zinc deficiency (Ahmed *et al.*, 2014). The results of a study conducted by Idris and Rehman (2005) in Abbottabad (Pakistan) showed 68% people affected by IDA. Another study done in Hyderabad in 2003-2005 revealed 90.5% prevalence of IDA among pregnant women (Baig-Ansari *et al.*, 2008). According to estimates of the National Nutrition Survey (NNS, 2011), 61.9% of 0-5 year old children had anaemia while 43.8% had iron deficiency in Pakistan. Pregnant women also suffered with widespread micronutrient deficiencies: anaemia 51.0%, IDA 37.0% and zinc deficiency 47.6%. Among non-pregnant women, the deficiency of micronutrients was as follows: anaemia 50.4%, IDA 26.8% and zinc deficiency 41.3% (NNS, 2011). Iron deficiency anaemia in Pakistan has shown a gruesome effect on maternal mortality rate, reaching 1578/100,000 live births in Karachi (Sheikh *et al.,* 2010). About 45% of overall prevalence of IDA in Pakistan has been reported by Mawani *et al.* (2016) based on recent statistics, indicating the current failure of public health measures to control IDA and requiring immediate attention of government bodies and NGOs. IDA can be significantly coped with appropriate cost-effective interventions taken on time.

Considering the significance of iron for human nutrition, huge consumption of these legumes in Pakistan and other developing countries and a sharp increase in the number of iron deficiency anaemia cases, present study was aimed to evaluate the variations in the iron concentration among different varieties of fresh beans such as cluster beans, peas, green chick peas, French beans and hyacinth beans. Some dry beans (chick peas) of different sizes were also analyzed to compare iron content. Estimating the iron levels in beans is momentous, mainly for the low-income population that is suffering from deficiency of this micronutrient. This study would indicate most appropriate food beans with high level of iron that could improve their nutritional status. The factors that can affect iron availability in beans have also been tracked.

MATERIALS AND METHODS

Reagents and Solutions:

All the reagents utilized in this work (1,10-phenanthroline, hydroxylamine hydrogen chloride, sodium acetate, ferrous ammonium sulphate, nitric acid, hydrochloric acid and ethanol) were of analytical grade and obtained from Merck. The solutions were prepared or diluted in distilled-deionized water. ELGA Cartridge Type C114 was used to deionize the distilled water prior to prepare required solutions. The glassware was soaked (15 min) in concentrated HNO_3, then washed and rinsed multiple times with distilled-deionized water prior to use to circumvent metal contamination.

Collection, Pretreatment and Drying of Samples:

Fresh raw bean samples of five different types, viz., cluster beans (*Cyanosis tetragonoloba*), pea (*Pisum sativum*), green chick peas (*Cicer arietinum*), French beans (*Phaseolus vulgaris*) and hyacinth beans (*Lablab purpureus*), were collected from local market of different areas of Karachi city (10 different samples for each species) during January to April. Among dry beans, five samples of each of small and large type variety of chick peas/garbanzo beans and kidney bean (a variety of *Phaseolus vulgaris*, red and white) were obtained for iron analysis. After the samples were surface sterilized, moisture content was removed in a preheated oven at 110–120ºC for 6–7 h.

Oxidation and Acid Digestion:

About 5 g of each dried sample was accurately weighed using a digital weigh balance (Panther-USA) and acid digested as per the standard AOAC method (AOAC, 2000). At first, all the samples were charred on blue flame till the complete removal of hydrocarbons. These samples were then oxidized using 2 mL concentrated nitric acid (HNO_3). Oxidized samples were heated for 2 minutes to evaporate acid and then placed in a furnace at 500ºC for ignition. After complete ignition, grey or white ash containing metal oxides was obtained. To these ashed samples, 5 mL concentrated HCl was added and heated at low flame till complete dryness. Further, 2 mL concentrated HCl was added again, heated, and distilled water was added upto 100.0 mL. Insoluble particles if any were removed before dilution by filtration, and filter paper was properly washed with distilled deionized water to ensure that all metal chlorides were transferred to the flask.

Standard Assay for Iron Analysis:

In a 10.0 mL aliquot of the digested sample solution, 1.0 mL hydroxylamine hydrogen chloride solution (10% w/v) was added to convert all iron into ferrous form. After incubation of 5 minutes at room temperature, 5.0 mL sodium acetate solution (27.2 g/100 mL water) as buffer and 1.0 mL of 1,10-orthophenanthroline (0.1 g dissolved in ethanol and diluted to 100 mL with water) as complexing agent was added. The resulting solution was diluted upto 25.0 mL with distilled deionized water. The blank was run in the same way using the solvent alone. The absorbance of samples was measured at 510 nm using UV-visible spectrophotometer (Canadian Biomedical Company, 2800 software), and total iron content of the samples was determined using calibration curve method. The standard calibration curve (Fig. **1**) was drawn using standard solutions of Fe (II) of different concentrations (10–100 μM) prepared immediately before use from stock ferrous ammonium sulphate solution by similar treatment with reagents (hydroxylamine hydrogen chloride, sodium acetate and 1,10-phenanthroline) as mentioned above.

Statistical Analysis:

All the samples were run in 5 or more replicates and the results of iron content analysis were presented as the mean (mg/L of digested sample) ± SD values. Under applied conditions, x mg Fe/L of digested sample ≈ $2x$ mg Fe/100 mg dry beans. Data for calibration curve was subjected to regression analysis, and statistical analysis of data was done employing Minitab version 15 using ANOVA and student's *t* test at 5% level of significance (Quinteros *et al.* 2001; Carvalho *et al.*, 2012).

RESULTS AND DISCUSSION

As per the WHO estimates, 50% of women and 42% of the children suffer from iron deficiency anaemia worldwide (WHO, 2017). Usually, iron food interventions are prescribed to cope with iron deficiency. However, because of the low socioeconomic status, considerably large proportion of population in the developing countries cannot afford iron fortified food or interventions (Stoltzfus, 2011; Mawani *et al.*, 2016). In addition, a number of reports mentioned the increased morbidity level with these iron interventions particularly in immuno-compromised children owing to cause disturbance in the gut microbiome and thus can enhance gut inflammation (Baumgartner and Barth-Jaeggi, 2015). Thus, emphasis should be focused on the use of cheap natural resources enriched in iron. Considering this, the present research work was based on the analysis of iron in different types of beans using an economic, easy and highly sensitive spectrophotometric method.

For each fresh bean species ten replicates were performed. The mean, standard deviation, and 95% confidence limits are reported in Table 1. Analysis of variance was performed to evaluate the difference in the iron content (Table 2). One-Way ANOVA was used to analyze the significance of variance due to differences in iron content of the selected species from random variation of replicate analysis. The iron content in the selected species was assumed same as per null hypothesis. The F-value, 25.52, was calculated using mean squares for factors (beans species) and error (random error). The $P = 0.000$ concluded that the null hypothesis is rejected and the iron content in selected fresh bean species was significantly different. The mean iron content in different fresh beans was found in the range of 4.632 ± 0.7322 to 10.907 ± 1.7558 mg/100g dry bean (x mg/L ≈ $2x$ mg/100 mg). The observed range is closer to reported ranges of iron for different legumes (Carvalho *et al.*, 2012; Brigide *et al.*, 2014). The iron content in beans may vary due to several factors that have been discussed later in this section.

The iron content of peas, cluster beans, French beans, and hyacinth beans was similar. The conclusion is based on the overlap of data distribution. The iron content of fresh green chick peas (2.3160 ± 0.3661 mg/L ≈ 4.632 ± 0.7322 mg/100g) is significantly lower than rest of the selected varieties (Table 1). Quinteros *et al.* (2001) observed iron content of 5.45 ± 0.05 mg/100g in chick peas, which is slightly higher than our results for chick peas. The lowest mean iron content in chick peas and then in peas may be because of high dietary fibers in them, which may reduce the availability of minerals (Spiller, 2001). French beans contain vitamin A and C which help facilitating iron mobilization from its storage sites and its absorption, respectively (Gleerup *et al.*, 1995; Kolesteren *et al.*, 1999). Although, hyacinth beans contain calcium and zinc which resist the iron absorption, the high iron content in these beans might be because of the presence of vitamin C which increases the absorption of iron (Gleerup *et al.*, 1995; Moriyama and Oba, 2008; Saunders *et al.*, 2013). The highest average iron content found in cluster beans (5.4535 ± 0.8779 mg/L) may be because of its thicker covering or peel compared to others; cluster beans were analysed along with covering as these are consumed as a whole for food, while other fresh beans were tested after peeling off the covering in the way as they are consumed. It may also be due to high ability of cluster beans to make crosslinks with other metal ions that may resist the absorption of iron, thus making iron to be available in high amount.

Table 1. Amount of iron in different fresh beans.

Beans	*N*	Mean[a]	St.Dev.	95% Cls
Peas	10	4.4135	0.5106	4.049–4.778
Green Chick Peas	10	2.316	0.3661	2.054–2.578
Cluster Beans	10	5.4535	0.8779	4.826–6.081
French Beans	10	4.8649	0.9349	4.197–5.533
Hyacinth Beans	10	4.9672	0.3301	4.731–5.203

[a] Iron content in mg/L (x mg/L ≈ $2x$ mg/100 mg dry bean).

Table 2. Analysis of variance in iron content among different species of fresh beans.

Source	*Df*	*SS*	*MS*	*F*	*P*
Factor	4	44.959	11.240	25.52**	0.000
Error	35	15.413	0.440		
Total	39	60.373			

***P*<0.01

Table 3. Two sample t-test for small chick peas *vs.* large chick peas.

Type of Chick Peas	*N*	Mean	StDev	SE Mean	*t* value	*P*
Small Chick Peas	5	2.421	0.244	0.11	3.21*	0.016
Large Chick Peas	5	2.0577	0.0682	0.030		

**P*<0.05

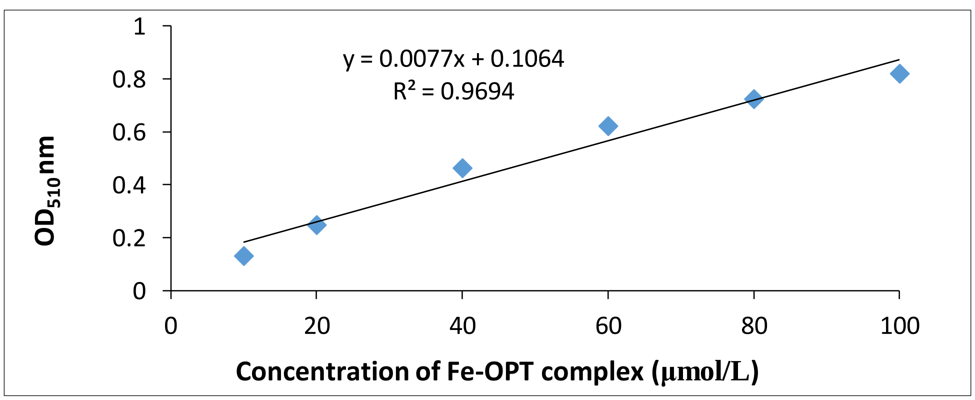

Fig. 1. Calibration curve for Fe-orthophenanthroline (Fe-OPT) complex.

Among dry beans, two different sized cultivars (small and large) of chick peas were selected for study, and student's *t*-test was used to evaluate the difference in the iron content between them. For this purpose, five replicates were performed for each bean type. The null hypothesis was that the mean iron content for the two cultivars is same. The mean iron content and its standard deviation are reported in Table 3. The *t*-test with different variances yielded $t = 3.21$, degree of freedom = 4, and $P = 0.016$. The null hypothesis was rejected due to *P*-value less than 0.05, and the mean iron content of small chick peas (2.4211 ± 0.244 mg/L) is not statistically similar to mean iron content of large chick peas (2.0577 ± 0.0682 mg/L). The iron content in fresh (green) chick peas was found significantly higher ($P<0.000$) compared to that in dry chick peas, showing that the fresh beans are better source of iron as compared to dry beans. This difference in iron content may because of different variety, storage time, some environmental contamination effects, and the way they have been preserved or stored (Carvalho *et al.*, 2012). Iron concentrations in beans can vary due to improper storage in the market or shops, particularly warm and moist conditions can change physiology and biochemistry in seeds that may affect nutrient bioavailability and nutritional quality of beans (Meyer *et al.*, 2013). Regardless of least iron content found for dry chick peas in our results, Huang *et al*. (2014) established the role of chickpea seed ferritin as a supplement to control iron deficiency anaemia.

The average iron content in different types of selected dry kidney bean (a variety of common bean *Phaseolus vulgaris*) was found to be 3.6409 ± 0.2768 mg/L, 3.5334 ± 0.4906 mg/L, 2.4560 ± 0.3765 mg/L and 2.3609 ± 0.2463 mg/L in small white kidney beans, large white kidney beans, small red kidney beans and large red kidney beans, respectively. Data shows that white and small kidney beans generally give higher iron content as compared to that given by red and large kidney beans, respectively. The higher iron content in white beans is due the fact that these beans contain more than 90% of total iron as ferritin or other soluble iron, which may be resistant to iron absorption inhibitors, providing better absorption of iron (Messina, 2014).

The data was also analysed pertaining to the sampling sites. Interestingly, it was observed that the samples which were collected from polluted areas or the areas situated near the petrol pumps, such as Sadder, Tariq Road and Grumander, generally contained less amount of iron in comparison to the other areas situated far away from petrol pumps or high traffic roads, such as Buffer Zone, Surjani Town and Gulberg. This may be because of high lead concentration in the atmosphere near petrol pumps or high traffic areas, which can contaminate food/vegetables and reduces the iron absorption (Kohno *et al.*, 1993; Makokha *et al.*, 2008). Pollutants may also function as chelators to minerals and can reduce their absorption in food samples (Schreinemachers and Ghio, 2016). Therefore, in polluted areas the green beans or other vegetables must be properly stored and preserved.

This study confirms that the beans, mainly cluster beans, are the excellent source of iron that can economically fulfill the daily iron requirement for the human body (14 mg/day) with many other nutrients. Therefore, the growth and consumption of these beans can be mentioned to overcome IDA, particularly in low-income populations of developing and underdeveloped nations, including Pakistan.

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