Research Article

Effect of iron fortified ready to drink beverage in combating anemia

Amal Shaukat¹, Muhammad Nadeem^{1*}, Arshad Mahmood^{2,3}, Zafar Iqbal Khan⁴, Mian Anjum Murtaza¹, Ahmad Din⁵, Mian Nadeem Riaz⁶, Lala Rukh¹, Hira Fatima^{4,7} and Ghulam Mueen-ud-Din¹

1. Institute of Food Science and Nutrition, University of Sargodha, Sargodha-Pakistan

2. Beijing Advance Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University, Beijing-China

3. Beijing Engineering and Technology Research Center of Food Additives, School of Food Science and Chemical Engineering, Beijing Technology and Business University, Beijing-China

4. Department of Botany, University of Sargodha, Sargodha-Pakistan

5. National Institute of Food Science & Technology, University of Agriculture, Faisalabad-Pakistan

6. Process Engineering R&D Center, Texas A&M University, College Station, Texas-USA

7. Department of Botany, University of Lahore, Sargodha Campus-Pakistan

*Corresponding author's email: mnadeemft@gamil.com

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Abstract

Food based approach has emerged as a suitable technology to combat micronutrient disorders. Food fortification with iron is the best strategy to combat iron deficiency anemia (IDA). The effect of an iron fortification can be judged through efficacy studies with improvement in the iron status of the target subjects. The purpose of conducting this study was to find out the effect of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in combating IDA among the female students of BS 6th Semester at Institute of Food Science and Nutrition, University of Sargodha, Sargodha. A preliminary demographic survey of 100 students was conducted and Hb level of 50 students were checked at University Medical and Diagnostic Center, UOS, Sargodha. Forty volunteer students (19-22 Y), who were anemic ($\text{Hb} < 12 \text{ g/dL}$) were enrolled for the efficacy study. One group (A) received ready to drink beverage without fortification, while the other group (B) received fortified drink containing 40 ppm $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ for a period of 3 months. The overall increase in Hb level was from 9.85 to 9.98 g/dL in group (A) while it was 10.53 to 11.52 g/dL in group (B) after 3 months. In case of storage and treatments, the observed difference was significant ($p > 0.05$) and highly significant ($p \leq 0.01$) respectively. It is concluded that ready to drink beverage is good vehicle for iron fortification and also a source of utilization of seasonal fruits and vegetables.

Keywords: Anemia; Efficacy; Fortification; Iron; RTD

Introduction

The deficiency of Iron is the main cause of anemia [1] and it is caused due to insufficient iron required in normal red blood cells formation and it typically results by severe blood loss, reduced intake of iron,

or both. Production of diminished red blood cells occurs in the body due to low iron stores which cause iron deficiency anemia [2, 3]. Anemic patients might suffer with light headedness, dyspnea and weakness; though, mild anemia may possibly produce

slight in the way of clinical signs and symptoms [4].

The anemia caused by deficiency of Iron in all age groups, is a main nutritional problem in Pakistan [5]. In reproductive age women, the anemia caused by deficiency of iron occurs up to 50% [6]. As a result, the rate of maternal mortality is high and in Pakistan. The major causes are found to be nutritional, i.e. Iron's dietary intake is not sufficient to fulfill the body's physiological needs. The other responsible factors are that iron bioavailability from cereal based foods is low, dietary practices are poor and people's purchasing power is low [5]. The single cost effective approach is food fortification using iron that can provide enduring improvement of iron [7]. Human diet must consist of variety of fruits. It has been usually reported that the possibility of chronic disease like cancer and cardiovascular disease can be decreased by consumption of diets loaded with fruit and vegetables [8, 9]. Peaches as an economic crop are therefore recommended for their numerous health benefits [10]. Many medicinal benefits are associated with peach because of the presence of important bioactive compounds [11]. In human nutrition, apricot has an important place and can be consumed fresh, dried or processed fruit. The high mineral contents of the apricot fruit make them helpful for treatment of bronchitis, blood poisoning, anemia, tuberculosis and asthma. The apricots are rich in iron content which make them useful in the fight against anemia and also consist of small amounts of copper, helps in absorption of iron in body. These fruits are antiseptic, emetic, ophthalmic and antipyretic [12]. The beetroot provides iron and vitamin C in addition to major nutrients including potassium and folate. Large quantity of biologically available antioxidants are present in beetroot juice [13] in addition to numerous health beneficial compounds like soluble fiber,

niacin, sodium, phosphorus, zinc, calcium, iron, folic acid and magnesium. In the juice of beetroot, the particular concern arises for the reason that high amount of polyphenol compounds are present in it [14]. To overcome the situation of anemia caused by deficiency of iron in Pakistan, fruit and vegetables based beverage of nutritional qualities was prepared. The objective of this project was to utilize seasonal fruits and vegetables for the development of ready to drink beverage fortified with iron to combat anemia through human subject.

Materials and methods

From the local bazar of Sargodha city, fully ripe seasonal fruits were purchased & the other materials and chemicals were purchased from the chemical store, Faisalabad. To remove dust and dirt, the fruits were washed and sorted properly. Fruit pulp was made after trimming and peeling of fruits. The juice extractor was used to extract juice. Pre-weighed ingredients were blended and chemicals were added for preparing drink. Two different formulations were prepared To (natural drink without fortificant) and T₁ (natural drink with fortificant) (Table 1).

Physico-chemical analysis of drink

The methods of AOAC [15] were used to find out total soluble solids. By the method No. 942.15 of AOAC [15], the juice acidity was determined. pH of the juices was measured by the method No. 981.12 as described in AOAC [15]. Non-reducing sugars, reducing sugars and total sugars and were measured by Lane and Enyon method in AOAC [15]. The concentration of sugar (°Brix) was divided by the concentration of citric acid to get the sugar/acid ratio of the drinks [16]. Method No. 42-11 of AACC [17] was used to determine total plate count. Method No. 42-50 of AACC [17] was used to find out mold/yeast count. The preparation of iron analysis sample was done by following the procedure of Duhan *et al.* [18]. The atomic absorption spectrophotometer (Model: Varian AA-240) was used to run the filtered sample solution. The iron sample

contents were measured by the respective standard curve prepared for each element [17].

Table 1. Formulation of fruit and vegetables based beverage

Ingredients	Quantity T ₀	Quantity T ₁
Apricot (g)	100	100
Peach (g)	80	80
Sugar (g)	100	100
Beetroot juice (ml)	20	20
Water (ml)	800	800
CMC (g)	1	1
Citric acid (g)	1	1
Sodium benzoate (g)	1	1
Ferrous sulphate FeSO ₄ .7H ₂ O (g)	-	0.1628

Efficacy study

This randomized clinical trial was done at the Institute of Food Science and Nutrition, University of Sargodha, Sargodha. Female students of 20–22 years of age were selected for this study. The protocol was reviewed and its approval was done by the Ethical Committee from the National Institute of Food Science & Nutrition, University of Sargodha. The students were randomly assigned to consume 200 mL/day of freshly prepared drink either fortified with 0.162g/L of ferrous sulphate.

Every student went through a thorough examination of blood like liver function test (LFT), renal function test (RFT), hemoglobin test and complete blood count (CBC). They also went through physical examination which includes body mass index (BMI), weight, height, pulse rate, blood pressure and body fat composition analysis using the Tanita TBF-410 Body Composition Analyzer (Tanita Corp., Tokyo, Japan).

Twenty healthy subjects (female) and twenty subjects with anemia (female) were enrolled for the study. Students were instructed to avoid unnecessary vigorous activities and to take normal diet. Blood samples were obtained from all subjects for liver function test (LFT), renal function test (RFT), hemoglobin test and blood count

(CBC). These tests were performed during the beginning and at the end of study period of three months.

Statistical analysis

The obtained data for different parameters was statistically analyzed for finding the significance level among quality parameters of various ready to serve drinks with the help of CRD (complete randomized design) and two factor factorial design and means were compared [19]. The significance level was set to be at $p \leq 0.05$.

Results and discussion

Ready to drink beverage was prepared by utilizing seasonal fruits and vegetables like apricot, peach and sugar beet. Fruit pulp and beet juice was blended and fortified with ferrous sulphate. Physico-chemical and microbiological analysis of the drinks was done. The analysis of drinks was also done for their sensory characteristics to assess their suitability by an untrained panel of judges. Clinical nutritional study also carried out on human subjects for three months. The results are explained and discussed below.

Physico-chemical analysis

Titrateable acidity

The mean values of acidity were $0.39 \pm 0.03\%$ and $0.43 \pm 0.03\%$ for T₀ and T₁ respectively. The range of mean values for treatments was from 0.39 ± 0.03 to $0.43 \pm 0.03\%$ having high acidity in case of

T₁ (0.46%) and low in T₀ (0.39%). The change in acidity might be due to the addition of ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$). The increasing acidity might be because of the reason that the amount of weekly ionized acids and acid's salts increases in their storage duration. The acid formulation occurs by the oxidation of reducing sugars, breakdown of pectin substances and degradation of polysaccharides which causes the acidity to rise (Figure 1).

Total soluble solids and pH

The mean values of TSS were 12.09 ± 0.08 and 12.14 ± 0.04 for T₀ and T₁ respectively.

The pH mean values for ready to serve drinks are presented in the (Figure 2 & 3). The mean values for pH were 4.16 ± 0.09 and 3.91 ± 0.09 for T₀ and T₁ respectively. For treatments, the mean values ranged from 4.16 ± 0.09 and 3.91 ± 0.09 having high pH in case of T₀ (4.16) and low in T₁ (3.91). The reason of change in pH of ready to drink beverage might be the change in acidity level of fruits and addition of citric acid and ferrous sulphate. The increase in acidity may be the cause of decrease in pH because pH and acidity are inversely related with each other.

Figure 1. Mean values of titrate able acidity in ready to drink beverage

Figure 2. Mean values of TSS in ready to drink beverage

Figure 3. Mean values of pH in ready to drink beverage

Determination of sugars

The mean values for reducing sugars were 1.56 ± 0.09 (T₀) and $1.6 \pm 0.07\%$ (T₁), non-reducing sugars were 10.36 ± 0.31 (T₀) and $10.35 \pm 0.19\%$ (T₁) and total sugars were 11.92 ± 0.27 (T₀) and $11.95 \pm 0.19\%$ (T₁) in RTS drink (Figure 4-6). The results are similar to the findings of Bajwa *et al.* [26]. The similar results were also observed by Nilugin and Mahendran [27] in a study related to preparation of RTS beverage from palmyrah pulp.

Sugar/Acid Ratio

It was measured by divided the

concentration of sugar (°Brix) with the concentration of citric acid (acid value) to find the drink's sugar/acid ratio. The analysis of variance indicated that treatment differ significantly for their sugar acid ratio ($p \leq 0.05-0.01$). The mean values for sugar/acid ratio were 31.15 ± 2.57 and 26.75 ± 1.53 for T₀ and T₁ respectively. The mean values for treatments ranged from 31.15 ± 2.57 to 26.75 ± 1.53 (Figure 7). The sugar/acid ratio was greater in case of T₀ (31.15 ± 2.57) as compared to T₁ (26.75 ± 1.53).

Figure 4. Mean values for reducing sugar content (%) in ready to drink beverage

Figure 5. Mean values for non-reducing sugar content (%) in ready to drink beverage

Figure 6. Mean values of total sugar content (%) in ready to drink beverage

Figure 7. Mean value for sugar/acid ratio in ready to drink beverage

Iron content

The mean values for iron content were 7.15 ± 0.26 (ppm) and 40 ± 1.26 (ppm) for T₀ and T₁ respectively (Figure 8). The mean values for treatments ranged from 7.15 ± 0.26

(ppm) and 40 ± 1.26 (ppm). The highest iron concentration was found in case of T₁ (40 ppm) while the minimum iron content was found in T₀ (7.15 ppm).

Figure 8. Iron content (ppm) mean values in ready to drink beverage

Microbiological analysis

The total plate count average mean values at 1st day of storage for T₀ and T₁ was 2.17 log₁₀ cfu/mL and after 90 days of storage, the TPC mean value for T₀ and T₁ was 2.34 log₁₀ cfu/mL. The TPC was more in case of T₀ (2.28 log₁₀ cfu/mL) while TPC was less in T₁ (2.22 log₁₀ cfu/mL). The change in TPC of beverage might be due to untreated

fruit pulp used for making ready to drink beverage. Pulp may be contaminated with bacteria, yeast and mold. The data depicts the minimum increase in microbial load after storage interval which might be due to initial load and untreated fruit pulp. However, during storage all treatments were found acceptable (Table 2).

Table 2. Mean value for TPC (log₁₀ cfu/mL) of ready to drink beverage during storage

Treatment	Storage (Days)				Mean
	0	30	60	90	
T ₀	2.20	2.25	2.29	2.39	2.28
T ₁	2.15	2.21	2.23	2.31	2.22
Mean	2.17	2.23	2.26	2.34	

The mold count was more in case of T₀ (1.87 log₁₀ cfu/mL) while less mold count was found in T₁ (1.75 log₁₀ cfu/mL). The change in MC of beverage might be due to untreated fruit pulp used for making ready to drink beverage (Table 3). Pulp may be

contaminated with yeast and mold. The data depicts the minimum increase in microbial load after storage interval which might be due to initial load and untreated fruit pulp. However, during storage all treatments were found acceptable.

Table 3. Mean values for mold count (log₁₀ cfu/mL) of ready to drink beverage in the storage period

Treatment	Storage (Days)				Mean
	0	30	60	90	
T ₀	1.76	1.84	1.92	1.97	1.87
T ₁	1.61	1.71	1.80	1.87	1.75
Mean	1.68	1.78	1.86	1.92	

Sensory analysis

Color's high score was obtained by the judges in case of T₀ (7.30) whereas color's less score was obtained in case of T₁ (6.96). Different coloring pigments of the fruit and addition of FeSO₄·7H₂O in beverage, causes the change in color of RTS beverage. However, the color score achieved for both treatments was acceptable. The high flavor score was achieved in case of T₀ (7.40) whereas less flavor score was obtained for T₁ (6.70). Beverage flavor may be changed because of the addition of FeSO₄. Beverage flavor is improved by the pH. However, acceptable score of flavor was achieved from both treatments. The score of taste was more for T₀ (7.46) whereas the minimum score for taste was obtained for T₁ (6.46).

Taste of drink was changed may be due to the addition of FeSO₄ as well as change in acidity of ready to drink beverage. However, acceptable score for taste was achieved for both treatments. The mouth feel score exceed in case of T₀ (7.36) as compared to T₁ (6.53). The change in mouth feel of ready to drink beverage might be due to addition of FeSO₄·7H₂O and change in acidity. In case of T₀, the high score of overall acceptability was given by judges (7.66) whereas less score of overall acceptability was observed for T₁ (6.86). The variation in overall acceptability of ready to drink beverage may be because of the decrease in color and flavor. However acceptable score was achieved for both treatments for overall acceptability (Figure 9).

Figure 9. Sensory score mean values for ready to drink beverage

Efficacy study of iron fortified drink

In the present scenario, food based approach has emerged as a suitable technology to combat micronutrient disorders [20]. In order to combat with the existing situation of iron deficiency anemia in Pakistan, fortification of food is the best strategy [21] and for this purpose food fortified with iron must be stable [22, 23] safe, acceptable and consumable by the targeted subjects [24]. The effect of an iron fortification can only be judged through efficacy studies with

improvement in the iron status of the target subjects. Hemoglobin (Hb) level is the key factor for the efficacy study. To determine the degree of anemia, important parameter is Hb level [25].

A preliminary demographic survey of 100 female students was conducted. During this survey, Hb level in blood of 50 females student was checked at University Medical and Diagnostic Center, University of Sargodha, Sargodha. Out of 50, forty volunteer students (19-22 Y), who were

anemic (Hb < 12 g/dL) were enrolled for the efficacy study (Table 4). One group received ready to drink beverage without fortification, while the other group received fortified drink containing 40 ppm FeSO₄.7H₂O for a period of 3 months. A few of the subjects have hemoglobin level (Hb) <8 g/dL while more fell in the group between Hb level of 8-9 g/dL (Table 5). After 3 months, the overall increase in level of Hb was from 9.85 to 9.98 g/dL in case of

drink without fortification while it was 10.53 to 11.52 g/dL after 3 months in case of drink with fortification. Highly significant ($p \leq 0.01$) variance was achieved in case of treatments and storage also has significant ($p > 0.05$) effect. The increase in Hb in case of FeSO₄.7H₂O fortified drink was of 0.094 g/dL while in non-fortified drink was 0.0132 g/dL. 9.40% and 1.32% reduction in iron deficiency was observed in these trials (Table 6 & 7).

Table 4. Mean values for Hemoglobin level in females during 90 days

Treatment	Days		Mean
	1	90	
T0	9.85	9.98	9.915b
T1	10.53	11.52	11.025a
Mean	10.19a	10.75b	

Table 5. Initial hemoglobin level in females

Age groups (years)	Hemoglobin (Hb) level (mg/dL)		
	<8	8-12	>12
16-25 (n=40)	0	55	24

Table 6. Hemoglobin level of females before and after taking drink without fortification

Variables	Control Treatment	
	Initial Hb level before studies Mean $\pm$ SD (n, %)	Final Hb level after studies Mean $\pm$ SD (n, %)
Haemoglobin (g/dL)	9.85 $\pm$ 1.27 (20, 100%)	9.98 $\pm$ 1.13 (20, 100%)
Iron Deficient (Hb is less than 11 g/dL)	9.48 $\pm$ 1.12 (16, 80%)	9.69 $\pm$ 1.05 (16, 80%)
Non-Iron Deficient (Hb is greater than 11 g/dL)	11.33 $\pm$ 0.47 (4, 20%)	11.15 $\pm$ 0.50 (4, 20%)

Table 7. Hemoglobin level of females before and after taking drink with fortification

Variables	Experimental Treatment	
	Initial Hb level before studies Mean $\pm$ SD (n, %)	Final Hb level after studies Mean $\pm$ SD (n, %)
Hemoglobin (g/dL)	9.97 $\pm$ 1.35 (20, 100%)	10.50 $\pm$ 1.06 (20, 100%)
Iron Deficient (Hb is less than 11 g/dL)	9.53 $\pm$ 1.12 (10, 50%)	10.89 $\pm$ 1.10 (10, 50%)
Non-Iron Deficient (Hb is greater than 11 g/dL)	11.53 $\pm$ 0.45 (10, 50%)	12.15 $\pm$ 0.54 (10, 50%)

Conclusion

From the above results / discussion, it is concluded that seasonal fruit are suitable as a source of iron for anemic patients. The ready to drink beverage is good vehicle for iron fortification and also a source of utilization of seasonal fruits. Food fortification is a practical approach to reach at the risk segments of the population at minimal cost. So, the iron deficiency anemia can be controlled with the help of consumption of fortified beverages that are very common in our society. It is helpful to increase the nutrient intake of children and their nutritional status. Beverages can be consumed without further processing/cooking, so it is termed as vehicle for fortification which is also easy to administer. To overcome the situation of iron deficiency anemia in Pakistan, a nutritious fruit and vegetables based beverage was prepared. The consumption of iron-fortified ready to drink beverage, using FeSO_4 as fortificant can effectively improve iron status, thereby, reducing iron deficiency.

Authors' contributions

Conceived and designed the experiments: M Nadeem, A Mahmood & ZI Khan, Performed the experiments: A Shaukat & L Rukh, Analyzed the data: MN Riaz, Contributed reagents/ materials/ analysis tools: MA Murtaza, A Din, H Fatima & GM Din, Wrote the paper: A Shaukat, M Nadeem & A Mahmood.

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