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Evaluation of physico-chemical properties of iron fortified yogurt

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Abstract
Hidden hunger is a chronic lack of vitamins and minerals that often has no visible warning signs. Worldwide, the three most common forms of micronutrient malnutrition are iron, vitamin A and iodine deficiency. Iron Deficiency Anaemia is one of the most common deficiencies people suffer from. Food fortification can lead to relatively rapid improvements in the micronutrient status of a population, and at a very reasonable cost. In the present study yogurt is used as a vehicle of fortification. Yogurt is a popular fermented dairy product. It is produced by the fermentation of milk by bacterial cultures known as yogurt cultures which contain strains of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus bacteria. Compared to milk, yogurt is more nutritious, but it contains very little amount of iron. In the present study yogurt was fortified with ammonium ferrous sulphate, ferrous sulphate, iron-casein complex in three different concentrations (20mg, 30mg, 40 mg iron) /kg milk sample. Yogurt samples were analyzed physicochemically at 1st, 4th, 7th day of storage. Physicochemical study shows a significant difference between different yogurt sample concentrations and storage period. The results suggest possibility of making good quality yogurt by fortifying milk with ammonium ferrous sulphate, ferrous sulphate and Iron casein complex.

Keywords: hidden hunger, anaemia, iron, fortification, yogurt, physicochemical, micronutrient, malnutrition

Introduction
Iron is one of the most essential trace elements. In 1713 AD, Lernery first recognized iron as a constituent of human body and in 1800 AD, Lecanu identified iron in the metalloprotein haemoglobin. Now it is known that, in the body, all the iron exist in combination with protein molecules. The overall iron content of human body is about 2.5 to 4.0g. Although the amount varies in individuals depending on age, sex, body stature, nutritional status, general health and level of iron stores [1].

Most of the iron in the body is contained within haemoglobin, an erythrocyte protein that carries oxygen from the lungs to the tissues. Iron is an essential component of myoglobin, a protein that carries oxygen to the muscles. Iron is also essential for growth, development, normal cellular functioning, and synthesis of some hormones and connective tissue [2]. Iron deficiency is the most common nutritional deficiency in poor countries of the developing world. About 50% of these iron deficient individuals develop Iron Deficiency Anaemia (IDA). The World Health Organization (WHO) ranked iron deficiency as 7th out of 10 major preventable risks for diseases and death that together responsible for 40% of the 56 million deaths that occur world-wide each year [3]. Iron Deficiency Anaemia can lead, reduced work capacity in adults and impact motor and cognitive development in children and adolescents. There is some evidence showing that iron deficiency without anaemia affects cognition in adolescent girls and causes fatigue in adult women. IDA may affect visual and auditory functioning and is weakly associated with poor cognitive development in children [4]. Food fortification or enrichment refers to the process of adding micronutrients (essential trace elements and vitamins) to food to improve its quality or to deliver that micronutrient to the population to correct the on-going nutritional deficiency among them [5].

According to the FAO and WHO, Food fortification was identified as one of the important strategy to decrease the incidence of nutrient deficiencies globally [6]. Yogurt is a fermented milk produced by Streptococcus thermophilus and Lactobacillus bulgaricus spp. Compared to milk, yogurt is more nutritious and is an excellent source of protein, calcium, phosphorus, riboflavin, thiamine, vitamin B12, folate, niacin, magnesium and zinc. Since lactose in milk is converted to lactic acid during Fermentation and due to the presence of lactose fermenting bacteria in yogurt, lactose intolerant people can consume yogurt without any adverse effect. Consumption of fermented milk products causes a slight reduction in stomach pH which reduces the risk of pathogen transit and the effects of low gastric juice secretion problem. [7] Therefore dairy products are good for iron fortification because they have high nutritive value and reach targeted population. [8] The ideal iron compound used as fortificant should supply high bioavailability iron, it should not affect the physico-chemical properties of food product [9, 10]. The purpose of this study is to prepare iron-fortified yogurt with ammonium ferrous sulphate, ferrous sulphate, Iron-casein complex at three different concentrations (20mg,30mg,40mg/kg milk) as it covers respectively 6.89%,10.34% and 13.79% of RDA of iron for an adult Indian woman if the person consumes 100gm of yogurt every-day. Yogurt samples were analyzed physico-chemically on 1st, 4th, 7th day of storage.

Materials and Methods

The study was designed to prepare Iron fortified yogurt and physicochemical analysis of all the combinations at 1st, 4th, 7th day of storage period.

Preparation of Iron fortified Yogurt: Locally available Amul Taja toned homogenized pasteurized milk was taken. Milk was fortified with ammonium ferrous sulphate, ferrous sulphate, Iron-casein complex. The milk was divided into ten portions. The first portion was not fortified with iron and regarded as control. The rest nine portions were fortified with ammonium ferrous sulphate, ferrous sulphate, Iron-casein complex in different concentration respectively 20mg, 30mg, 40mg iron/kg milk. Then milk was inoculated with yogurt culture and filled into previouly sterilized plastic cups, covered and kept at room temperature (35-36ºc) until a firm curd was formed (approximately 6-7 hours). The resultant yogurt was kept in a refrigerator for 7 days at 4ºc. [11].

Physicochemical analysis

1. Preparation of iron fortified yogurt (11).
2. Physicochemical analysis of iron fortified yogurt
1. Volume of whey by syneresis index (12).
2. Determination of Total Titratable Acidity (11).
3. Determination of Moisture content (13).
4. Determination of Total solid content (13).
5. Determination of Ash content (13).
6. Determination of pH (14).
7. Determination of viscosity (15).
8. Determination of Thiobarbituric acid (16).
9. Determination of Peroxide value (3).

Statistical analysis: For statistical analysis Duncan's test was done for all the parameters.

Results

Physicochemical Analysis of iron fortified yogurt

Table 1: Effect of using different iron salts on Volume of whey by syneresis index of iron fortified yogurt during storage period at 4(±2ºc)

Treatment	1st	4th	7th
NF	11.84a±0.17	11.80 a ±0.08	11.74 a ±0.08
AFS 20	11.82 a ±0.05	11.79 a ±0.06	11.75 a ±0.03
AFS 30	11.83 a ±0.03	11.81 a ±0.04	11.77 a ±0.06
AFS 40	11.81 a ±0.06	11.78 a ±0.04	11.73 a ±0.03
FS 20	11.80 a ±0.07	11.76 a ±0.03	11.71 a ±0.02
FS 30	11.82 a ±0.02	11.79 a ±0.04	11.76 a ±0.03
FS 40	11.83 a ±0.04	11.80 a ±0.02	11.76 a ±0.04
IC 20	11.80 a ±0.03	11.77 a ±0.03	11.75 a ±0.01
IC 30	11.84 a ±0.03	11.82 a ±0.03	11.79 a ±0.02
IC 40	11.82 a ±0.04	11.80 a ±0.03	11.77 a ±0.01

Table 2: Effect of using different iron salts on Total titratable acidity of iron fortified yogurt during storage period at 4(±2ºc)

Treatment	1st	4th	7th
NF	0.41 a ±0.03	0.42 a ±0.01	0.44 a ±0.02
AFS 20	0.42 a ±0.02	0.43 a ±0.02	0.44 a ±0.01
AFS 30	0.40 a ±0.01	0.42 a ±0.03	0.44 a ±0.01
AFS 40	0.41 a ±0.01	0.42 a ±0.02	0.43 a ±0.01
FS 20	0.41 a ±0.02	0.42 a ±0.03	0.44 a ±0.01
FS 30	0.42 a ±0.01	0.43 a ±0.01	0.44 a ±0.01
FS 40	0.43 a ±0.02	0.45 a ±0.01	0.46 a ±0.02
IC 20	0.40 a ±0.03	0.42 a ±0.03	0.43 a ±0.02
IC 30	0.41 a ±0.01	0.42 a ±0.02	0.43 a ±0.01
IC 40	0.40 a ±0.02	0.41 a ±0.02	0.43 a ±0.01

Table 3: Effect of using different iron salts on Moisture of iron fortified yogurt during storage period at 4(±2ºc)

Treatment	1st	4th	7th
NF	88.5 a ±0.06	88.48 a ±0.01	88.46 a ±0.02
AFS 20	88.48 a ±0.03	88.47 a ±0.03	88.45 a ±0.02
AFS 30	88.49 a ±0.02	88.47 a ±0.02	88.45 a ±0.01
AFS 40	88.5 a ±0.06	88.49 a ±0.01	88.47 a ±0.02
FS 20	88.47 a ±0.02	88.44 a ±0.01	88.42 a ±0.03
FS 30	88.48 a ±0.07	88.46 a ±0.03	88.44 a ±0.02
FS 40	88.49 a ±0.03	88.47 a ±0.01	88.45 a ±0.02
IC 20	88.47 a ±0.02	88.48 a ±0.02	88.5 a ±0.01
IC 30	88.46 a ±0.03	88.47 a ±0.02	88.49 a ±0.03
IC 40	88.48 a ±0.07	88.49 a ±0.03	88.5 a ±0.02

Table 4: Effect of using different iron salts on Total solid of iron fortified yogurt during storage period at 4(±2ºc)

Treatment	1st	4th	7th
NF	11.5 a ±0.06	11.52 a ±0.01	11.54 a ±0.02
AFS 20	11.52 a ±0.03	11.53 a ±0.03	11.55 a ±0.02
AFS 30	11.51 a ±0.02	11.53 a ±0.02	11.55 a ±0.01
AFS 40	11.5 a ±0.06	11.51 a ±0.01	11.53 a ±0.02
FS 20	11.53 a ±0.02	11.56 a ±0.01	11.58 a ±0.03
FS 30	11.52 a ±0.07	11.54 a ±0.03	11.56 a ±0.02
FS 40	11.51 a ±0.03	11.53 a ±0.01	11.55 a ±0.02
IC 20	11.53 a ±0.02	11.52 a ±0.02	11.5 a ±0.01
IC 30	11.54 a ±0.03	11.53 a ±0.02	11.51 a ±0.03
IC 40	11.52 a ±0.07	11.51 a ±0.03	11.5 a ±0.02

Table 5: Effect of using different iron salts on Ash of iron fortified yogurt during storage period at 4(±2ºc)

Treatment	1st	4th	7th
NF	0.68 a ±0.02	0.67 a ±0.01	0.65 a ±0.02
AFS 20	0.69 a ±0.01	0.67 a ±0.01	0.66 a ±0.02
AFS 30	0.70 a ±0.02	0.69 a ±0.03	0.67 a ±0.01
AFS 40	0.69 a ±0.03	0.68 a ±0.01	0.67 a ±0.02
FS 20	0.71 a ±0.01	0.69 a ±0.01	0.68 a ±0.02
FS 30	0.70 a ±0.03	0.68 a ±0.02	0.66 a ±0.02
FS 40	0.71 a ±0.02	0.70 a ±0.02	0.68 a ±0.01
IC 20	0.70 a ±0.01	0.69 a ±0.02	0.68 a ±0.01
IC 30	0.70 a ±0.02	0.68 a ±0.01	0.67 a ±0.02
IC 40	0.71 a ±0.01	0.70 a ±0.01	0.68 a ±0.02

Table 6: Effect of using different iron salts on pH of iron fortified yogurt during storage period at 4(±2ºc)

Treatment	1st	4th	7th
NF	4.10 a ±0.03	3.85 a ±0.03	3.41 a ±0.02
AFS 20	4.18 a ±0.02	3.81 a ±0.01	3.38 a ±0.01
AFS 30	4.10 a ±0.06	3.82 a ±0.02	3.4 a ±0.02
AFS 40	4.17 a ±0.04	3.81 a ±0.01	3.37 a ±0.02
FS 20	4.14 a ±0.06	3.82 a ±0.02	3.39 a ±0.03
FS 30	4.11 a ±0.01	3.84 a ±0.02	3.36 a ±0.02
FS 40	4.10 a ±0.02	3.83 a ±0.01	3.40 a ±0.02
IC 20	4.17 a ±0.03	3.89 a ±0.03	3.38 a ±0.04
IC 30	4.13 a ±0.04	3.87 a ±0.03	3.36 a ±0.03
IC 40	4.20 a ±0.02	3.90 a ±0.03	3.37 a ±0.02

Table 7: Effect of using different iron salts on Viscosity of iron fortified yogurt during storage period at 4(±2ºc)

Treatment	1st	4th	7th
NF	2167 a ±1	2169 a ±1	2170 a ±2
AFS 20	2164 a ±1	2166 a ±2	2167 a ±3
AFS 30	2169 a ±1	2170 a ±1	2172 a ±2
AFS 40	2164 a ±3	2165 a ±1	2167 a ±2
FS 20	2165 a ±2	2167 a ±1	2168 a ±2
FS 30	2164 a ±2	2165 a ±1	2167 a ±1
FS 40	2166 a ±1	2168 a ±2	2169 a ±1
IC 20	2168 a ±1	2169 a ±3	2170 a ±2
IC 30	2167 a ±2	2168 a ±1	2170 a ±1
IC 40	2166 a ±3	2167 a ±2	2169 a ±1

Table 8: Effect of using different iron salts on TBA of iron fortified yogurt during storage period at 4(±2ºc)

Treatment	1st	4th	7th
NF	0.06 [a] ±0.01	0.07 [a] ±0.02	0.09 [a] ±0.01
AFS 20	0.07 [a] ±0.01	0.08 [a] ±0.02	0.10 [a] ±0.01
AFS 30	0.08 [a] ±0.02	0.09 [a] ±0.01	0.11 [a] ±0.02
AFS 40	0.07 [a] ±0.01	0.08 [a] ±0.01	0.09 [a] ±0.01
FS 20	0.08 [a] ±0.01	0.09 [a] ±0.02	0.11 [a] ±0.02
FS 30	0.07 [a] ±0.01	0.09 [a] ±0.02	0.10 [a] ±0.02
FS 40	0.07 [a] ±0.02	0.08 [a] ±0.01	0.10 [a] ±0.01
IC 20	0.08 [a] ±0.02	0.09 [a] ±0.01	0.10 [a] ±0.02
IC 30	0.09 [a] ±0.01	0.10 [a] ±0.01	0.11 [a] ±0.02
IC 40	0.08 [a] ±0.01	0.09 [a] ±0.02	0.11 [a] ±0.01

Table 9: Effect of using different iron salts on Peroxide value of iron fortified yogurt during storage period at 4(±2ºc)

Treatment	1st	4th	7th
NF	3.47 [a] ±0.04	3.49 [a] ±0.03	3.51 [a] ±0.02
AFS 20	3.49 [a] ±0.02	3.51 [a] ±0.01	3.53 [a] ±0.02
AFS 30	3.50 [a] ±0.03	3.51 [a] ±0.03	3.53 [a] ±0.02
AFS 40	3.52 [a] ±0.01	3.53 [a] ±0.01	3.56 [a] ±0.03
FS 20	3.51 [a] ±0.02	3.53 [a] ±0.03	3.54 [a] ±0.01
FS 30	3.53 [a] ±0.03	3.54 [a] ±0.03	3.55 [a] ±0.01
FS 40	3.50 [a] ±0.02	3.52 [a] ±0.02	3.54 [a] ±0.01
IC 20	3.51 [a] ±0.04	3.53 [a] ±0.02	3.54 [a] ±0.02
IC 30	3.54 [a] ±0.02	3.55 [a] ±0.01	3.56 [a] ±0.01
IC 40	3.53 [a] ±0.02	3.55 [a] ±0.02	3.56 [a] ±0.02

Discussion

Table 1 shows volume of whey non-significantly decreases as storage period increases. According to Dave, R.I. *et al*. (1988) [17] cited in Amira M.El-kholy *et al*. (2011) the syneresis decreased in all samples during storage as a result of improve the protein hydration of some of the free water. According to Achanta *et al*. (2007) [18] cited in Amira M.El-kholy *et al*. (2011) the volume of syneresis decreased due to fortified yogurt with iron improved the water holding capacity.

Table 2 Total Titratable Acidity non-significantly increases as period of storage increases. Similar results shows in Smith Gilliard Nkhata *et al*. (2015) [3], N. Askary *et al*. (2013) [19], Osman, M.M *et al*. (2004) [20]. The increase in acidity may be due to continued production of lactic acid by lactic acid bacteria during storage condition.

Table 3 shows as the storage period increases moisture content decreases but the value is statistically non-significant.

Table 4 shows total solid content increases during storage condition, which is statistically non-significant.

Table 5 shows result of ash contain statistically non-significant difference during storage period.

Table 6 shows overall no significant changes in pH value for different iron samples. This results are in agreement with Hekmat and McMahon (1977) [21] cited in Amira M.El-kholy *et al*. (2011) [11]

Table 7 shows viscosity non-significantly increases during storage condition. According to Sadler *et al*. (1973) [22] cited in Kim *et al*. (2002) viscosity of yogurt decreases during storage may be because an interaction of casein and whey protein with iron complex.

Table 8 shows TBA value shows non-significant changes during storage. Amira M.El-Kholy *et al*. (2011) [11] shows same result, the slight increase in oxidation may be due to the high acidity of yogurt which prevent or greatly reduce oxidation potency and formation of iron hydroxides.

Table 9 Peroxide value increases as storage period exceeds but it has no statistical significance. According to Smith Gilliard Nkhata *et al*.(2015) [3] iron fortification occurred by ferrous bisglycinate (63mg/kg), ferrous lactate (79mg/kg), ferrous sulfate (83mg/kg) and the peroxide value shows statistical significance result as storage period continues, it may be occurs due to use of higher quantity of iron fortificant during iron fortification significantly increases lipid oxidation. Ferros sulfate microencapsulated yogurt shows same result, however it may be concluded microencapsulation did not provide enough barrier between the iron and milk lipids such that oxidation occurred.

Conclusion

The result suggest possibility of making good quality yogurt by fortified yogurt milk with ammonium ferrous sulphate, ferrous sulphate, iron-casein complex in 20mg, 30mg, 40mg/Kg milk respectively. The study concludes that iron fortification does not affect the overall physicochemical properties of yogurt and the product may be used for prevention of iron deficiency anaemia.

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