FOOD PRODUCT DEVELOPMENT FROM FERRIC FRUCTOSE IRON FORTIFIED BOVINE MILK PREPARATION AND THEIR BIOAVAILABILITY APPRAISAL AS A SUITABLE SOURCE OF IRON.

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Abstract

Protein energy malnutrition and Iron Deficiency Anaemia is the twin maladies of dwindling resources and increasing population is galore in developing countries, India being no exception. Bovine milk fortified or combined with iron and vitamin C can become the most equipped nutritional weapon to combat not only anaemia and Protein energy malnutrition but a broad spectrum malnutrition eradicating food item encompassing the prevention and treatment of almost all the nutrition deficiency disorders. In this study, two complexes of ferric namely ferric fructose and ferric lactose were prepared using laboratory chemical synthesis and milk preparations were fortified with the former due to its high solubility and stability characteristics by using a system of iron and ascorbic acid. The process of product development was undertaken by using paneer made from fortified and unfortified bovine milk. Iron bioavailability appraisal was done by giving these food products to the anaemic subjects in fasting state. The results point towards the possibility of this food product to be a good source of iron and protein for the under nourished masses.

Keyword: Anaemia, Bovine milk, Ferric complexes, Iron fortification, Product development, malnutrition

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1. Introduction

Global malnutrition is a major area of concern for public health and encompasses a spectrum of deficiencies. Protein Energy malnutrition and iron deficiency anemia, the twin maladies of dwindling resources and increasing population are glore in developing countries, India being no exception. For the year 2011, it is estimated that roughly 43% of children, 38% of pregnant women, and 29% of nonpregnant women and 29% of all women of reproductive age have anaemia globally, corresponding to 273 million children, 496 million non-pregnant women and 32 million pregnant women. (WHO,2015). Malnutrition is a major health problem, especially in developing countries. It affects almost 800 million people. Prevalence rates vary among different continents of the world. PEM is undoubtedly the most serious nutritional problem affecting several thousand young children in India (Gaudappa and Divyarani,2015). The disease is of particular significance in preschool children and women because of high prevalence (50-70%) and the adverse functional consequences that it produces. where as in school children anemia impairs scholastic performance, it tells upon the reproductive performance in young women (Ramen at al 2010). Saibaba (1994) hails milk as the miracle complete food. According to rural population survey by NIN (1992), average incidence of anemia in general public is 58.7%. Serves conducted under the aegs of ICMR (2006) across the country has revealed that 62% pregnant women suffer from anemia. The chief factor responsible for this is inadequate intake through diet and poor availability. To overcome these we need to go for iron fortification and supplementation with suitable promoters. Keeping all these factor in mind the current study in essence is an endeavor to nutritionally strengthen milk by enriching with a new class of additives; the sugar iron complexes and evaluating the food product formed from it, as a matter of fact bovine milk despite being nutrient rich has its Achilles heels, the deficiency of iron and vitamin C. That is why it has been made to undergo processing metamorphosis by food scientist and food technologists in a bid to optimize on its acceptability and nutritive quality characteristics. Milk has been enriched using a suitable iron sugar complex for which a few iron sugar derivatives have been synthesized in the laboratory. Using iron enriched milk, food products have been tested for acceptability and iron bioavailability among adolescent anemic girls.

The study, though undertaken in simple manner, Iron enrichment has been affected, has gone to unfold some interesting facts that would enhance the nutritive potential of milk and go a long way in making these products cheap, easily available and effective source of dietary iron along with its mainstay nutrient protein.

2. METHODS AND MATERIALS

The present study comprised a multifarious but integrated approach with the experimental design consisting of various phases as given under:

2.1 Phase 1: Preparation of iron sugar complexes for fortification

Many iron sugar complexes were chemically synthesized in the laboratory like Ferric fructose and Ferric lactose etc. Ferric fructose was selected for further experimentation involving fortification and bioavailability appraisal due to high solubility and low or no colour impairing fall out.

Ferric fructose has been prepared by using methods given by Saltman (1963).

2.1.1 Ferric fructose was prepared by mixing equal volumes of 0.1 m ferric chloride and 1m fructose at room temperatures.

2.1.2 pH was adjusted to 11 with 1N NaOH

2.1.3 To the clear solution absolute ethanol (four times the volume of $FeCl3$ or fructose) was added to precipitate the complex from its sequestered solution.

2.1.4 Precipitates of ferric fructose were centrifuged and dissolved in the minimum amount of water.

2.1.5 PH was again adjusted to 11 and the complex was re-precipitated with ethanol.

2.1.6 Precipitates were separated by centrifugation and washed with alcohol followed by acetone.

2.1.7 Ferric fructose complex thus formed was dried over anhydrous $CaCl2$ at room temperature in a vacuum desiccator.

2.1.8 The complex was stored at 40℃ in brown bottles.

Ferric fructose thus prepared standardized by comparing with the commercial preparation of ferric fructose obtained from Siderplex manufactured by Raptakos Brett and Co. Ltd.

2.2 Phase 2: Fortification of milk with iron and ascorbic acid system

Milk was fortified with a system containing both iron and ascorbic acid (Kiran Ravi et al 1986). The method is as follows:

2.2.1 Ten ml water was added to 90 ml milk.

2.2.2 Milk + ferric fructose

Fifty mg ferric lactose dissolved in 10 ml of water was added to 90 ml of milk and a homogenous mixture was made by shaking in a vortex mixer. This amount of the iron complex was equivalent to 125 mg elemental iron per little of milk ≡125 ppm. It presented a much higher dose for human adults than the daily requirement (~2mg absorbed Fe 15-20 mg added Fe). Considering the daily intake of one bottle of milk (500ml)by an adult, it would amount to 60mg/day, which exceeds the daily requirement of 15-20mg Fe/day by a factor of 3. If this dose in milk is proved satisfactory, one third of this cost could certainly be beautiful.

2.2.3 Milk + ascorbic acid

50 mg ascorbic acid in 10 ml water was mixed with 90 ml milk. This represented a far higher dose of the vitamin in comparison to the daily human requirement. It was thought that this could certainly take care of all the possible losses of vitamins as well as afford data for the higher amounts needed for reasons explained in case of milk+ ferric fructose. Ascorbic acid utilized on account of being the only iron bioavailability enhancer from the vegetarian diet (Fidler et al, 2004).

2.2.4 Milk + ferric fructose + ascorbic acid

50 mg each of ferric fructose and ascorbic acid were dissolved in 10 ml water and 90 ml of milk was added to this mixture. It stirred in a vortex mixer.

Direct addition of ferric fructose to milk does not affect it but such an addition of ascorbic acid curdles the milk. It is therefore advisable to dissolve these compounds in an aliquot of water (10 ml) before adding to milk.

In all cases, heating at 79 to 81 $^{\circ}$C for half an hour resorted in order to attain complete pasteurization of milk samples.

2.3 Phase 3: Development and standardization of a milk product recipe for breakfast

The most suitable milk recipe named paneer noodles have been selected and standardized as follows:

2.3.1 1ml of ferric fructose solution contained 10 mg iron.
2.3.2 Milk was coagulated and whey water was collected.
2.3.3 The developed recipe was standardized in such a manner that the total amount of iron in single serving was 20 mg.
2.3.4 The soaking capacity of noodles for whey was standardized as 200ml whey water absorption by 60 g of noodles for a single serving containing 20 mg iron.
2.3.5 Organoleptic and sensory evaluation sessions of various trained and semi trained panel was done as per the specified standard. Procedure laid down for it by using triangle and hedonic tests.
2.3.6 pH was determined by using pH meter.
2.3.7 Moisture content was estimated by oven drying method.
2.3.8 For iron estimation muffle furnace ashing was done and suitable aliquots were used for estimation of iron.

2.4 Phase 4: Sampling

This study was conducted on university students of Banasthali Vidyapith in the age group of 20- 22 years. All were hostelers and come from same socioeconomic background. Ten students were selected as a part of study on purposive basis. The cut off point for blood haemoglobin level was 12g/dl for the anaemics as given by WHO (1972). Haemoglobin estimation was done by using cynomethaemoglobin method.

2.5 Phase 5: Bioavailability appraisal

All the subjects were not allowed to take food after 9p.m. and the venous blood was taken after 12 hrs i.e. at 9 a.m.

Breakfast containing 100 g Vitamin C solution (200 mg vitamin C/100 ml) and prepared recipe (2.3 Phase 3) provided to all human subjects immediately after drawing blood.

After 1 hour of breakfast the venous blood was taken again.

The same procedure was followed twice with fortified and unfortified bovine milk paneer (30 g/ serving) noodles recipe prepared from 200 ml whey water.

2.5.1 Preparation of serum

2.5.1.1 The blood from syringe is drawn in the dry acid cleaned voile.
2.5.1.2 It is left undisturbed for an hour to coagulate it.
2.5.1.3 The coagulated blood was centrifuged for 15 minutes.
2.5.1.4 2 ml of serum was pipetted out in separate 20 test tubes each tube was having demarcation.

2.5.2 Serum Iron Estimation

Bioavailability appraisal is done by estimating serum iron levels before and after the test breakfast using the method of Wongs (1928).

Serum proteins are precipitated with a reagent containing hydrochloric acid (to dissolve iron), thioglycolic acid (to reduce iron to ferrous state) and trichloro acetic acid (to precipitate proteins). the iron in ferrous state reacts with chromogen to give pink coloured complex which is measured colorimeterically.

3. Results and discussion

3.1Iron content of developed recipe

Milk procured from local vendor and the iron content of developed recipe have been calculated from ICMR nutritive value table and compared with the dietary reference values given by Hurrell and Egli (2010); as depicted in Table 1.

Table 1: Iron content of the developed recipe (one serving)

Ingredients in one serving	Amount (gm)	Iron content (mg)
Vermicelli	60	1.2
Pea	10	0.15
Onion	5	0.03
Cabbage	10	0.08
Total		1.46
Unfortified Bovine Milk paneer whey water	30	0.4
Fortified Bovine Milk Paneer and Whey water	30	20.4
Total iron content of Unfortified Bovine Milk paneer recipe	------	3.32
Total iron content of fortified Bovine Milk paneer recipe	------	23.32

The recipe was standardized and prepared by the method given by Showbhagya and Zakiruddin (2001)

***3.2* Sensory Evaluation:**

The combinations used for triangle test are as follows:

1. Unfortified bovine milk Paneer recipe
2. Fortified bovine milk Paneer recipe

Out of 10 panel members 8 could not differentiate between fortified and unfortified bovine milk paneer recipe. Providing indications that the ferric fructose complex fortified milk can become an acceptable tool in implementing iron fortification programs by selecting low cost iron salt with consumer acceptability and biological availability of iron.

Hedonic Test: To assess the overall acceptability of milk hedonic test was conducted and on the basis of the test scores recipe were designed as there was no significant difference in the scores and to utilize the milk protein to a good extent. Spices were added to impart taste to this tasteless product.

Thus Vermicelli paneer noodles were prepared with a high soaking capacity of 200ml/60gm so that all the whey water can be utilized. Moreover, the developed recipe was accepted successfully to be used as a breakfast dish by the panel members without hesitation.

Table 2: Hedonic Scores of acceptability of bovine milk paneer noodles

Attribute	Unfortified bovine milk paneer recipe	Fortified bovine milk paneer recipe
Appearance	7.2	7.2
Taste	7.1	7.2
After Taste	6.1	6.3
Colour	6.1	6.1

The scores seems to indicate that there was small change in acceptability in developed recipes due to fortification with ferric sugar complexes. Although bovine milk paneer recipe liked moderately.

3.3. Serum iron Response Test to Asses Bioavailability of iron:

Developed recipes provided to the subjects as a component of breakfast Miguel et al (2000) to assess the bio delivery potential of fortified and unfortified milk and its products. All the ingredients were kept quite identical quantitatively as well as qualitatively to obtain results without any nutrient lopsidedness.

Obviously due to low iron content (23.32mg in one serving) in the iron unfortified recipe. As clear from the table, the rise in serum level ranges between 2.74 to 11.88 µg/100ml. Process has been facilitated due to the intake of 200 mg vitamin C. The mean rise in serum iron level was 5.57 µg/100ml.

Table 3: Bioavailability appraisal from unfortified and fortified bovine milk paneer recipe and vitamin C solution on serum iron level

Subject	Serum iron level on unfortified bovine milk paneer recipe consumption		Serum iron level on fortified bovine milk paneer recipe consumption	
	Fasting serum iron level (µg\100ml)	Mean serum iron level after one hour of intake(µg/100ml)	Fasting serum iron level (µg\100ml)	Mean serum iron level after one hour of intake(µg/100ml)
T1	85.38	97.26	64.84	172.60
T2	80.36	85.38	80.36	97.26.
T3	105.93	108.67	105.02	128.31
T4	67.57	77.16	66.66	147.98
T5	105.02	111.41	105.02	129.22
T6	114.56	107.76	107.76	121.91
T7	115.98	119.63	55.70	74.42
T8	103.19	108.67	100.91	120.09
T9	92.23	97.26	118.26	137.44
T10	106.39	109.13	100.00	119.63

Unfortified bovine milk paneer recipe: Mean+S.E.M	95.58+6.87	101.466+6.04
Fortified bovine milk paneer recipe: Mean+S.E.M	77.97+11.29a	124.85+11.15a

The rise in serum iron level after taking the breakfast of given recipe with vitamin C solution was 31.14 µg/100ml indicating its potential of being an effective agent to cure iron deficiency anemia.

3.4. Statistical Analysis

The results were expressed as mean, SEM and significant difference was calculated by student's t test and paired t test method.

Table 5: Paired't'test value of rise in serum iron level after consumption of different recipes

Type of Recipe	Paired 't'test Value
Unfortified bovine milk paneer recipe	5.808a
Fortified bovine milk paneer recipe	5.54

The value of paired t test is significant at both levels. The rise in serum iron level magnitude was higher from fortified bovine milk paneer consumption.

Students 't' test applied to find out the significant difference between the unfortified and fortified milk paneer recipe consumption and found 4.83. Hence iron fortification has led to an increase in serum iron level of human subjects to a significant level.

4. CONCLUSION

- Iron sugar complexes can be prepared by simple laboratory methodology.
- Fortification by iron sugar complexes especially ferric fructose is by and large free from offensive odour and colour development.
- Acceptability and iron bioavailability of bovine milk paneer recipe is high to a significant extent.

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REFERENCES

1. Fidler, MC, Davidsson, L, Zeder C, &Hurrell ,RF.(2004).Erythobic acid is a potent enhancer of non heme-iron absorption. *Am J Clin Nutr*, 79-102.
2. Francisco et al. (2012). Milk Based Corn Starch Porridge Fortified with iron is Effective in Reducing Anemia. A Randomized Double blind Placebo controlled Trial.
3. Gopalan, C, Ramasatri, BV, balasubramanian, SC (2010). Nutritive Value of Indian foods, National institute of Nutrition, ICMR, Hyderabad, India.
4. Hurrell, richerd and egli, Ines (2010). iron availability and dietary references values. *Am J Clin Nutr*, 91:1461-7
5. JR, Hunt. (2005). dietary and physiological factors that affect the absorption and bioavailability of iron. *Int J Vitam Nutr Res*.,75(6):375-84.
6. Kiran, Ravi et al. (1977). Milk fortification with a system containing both iron and ascorbic acid. I*ndian journal of Nutrition and Dietetics.*
7. Kiran, Ravi, Kaur, Isht Preet &Vaneja, K. (1986). milk fortification with a system containing both iron and ascorbic acid. *Ind Jr of Nutr. And Dietet.*

8. Layrisse, Miguel, nieves, Maria Garcia –casal, Solano, liseti, baron, adela, maria, arguello, franklin, llovera, daisy, Ramirez, jose, leets, Irene & tropper eleonora. (2000). iron bioavailability in humans from breakfasts enriched with iron bis glycine chelate, phytates and polyphenols. *the journal of nutrition*,130: 2195-2199
9. Lindsay, W.L. and W.A., Norvell. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Amer. J.* 42:421-428.
10. Showbhagya, C.W., & Ali, Zakiruddin. (2001). Vermicilli noodles and their quality assessment. *Ind Jr of Food Sc. and tech*, 45:423-432.
11. Wongs. (1928) .The determination of iron and haemoglobin in Blood. *J. Biol. Chem*. 77: 499.
12. World Health Organization, Centers for Disease Control and Prevention. Worldwide prevalence of anaemia 1993–2005: WHO Global Database on Anaemia. Geneva: World Health Organization; 2008 (http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf, accessed 7 May 2015).
13. WHO. The global prevalence of anaemia in 2011. Geneva: World Health Organization; Printed by the WHO Document Production Services, Geneva, Switzerland,2015
14. International Journal of Contemporary Pediatrics *Patil GR et al. Int J Contemp Pediatr. 2015 Nov;2(4):307-310* http://www.ijpediatrics.com pISSN 2349-3283 | eISSN 2349-3291
15. Prevalence of protein energy malnutrition among 1-5 years of children in Bellary taluk DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20150924 Goudappa R. Patil, Divyarani D.C.