



Research Article

**IN-VITRO BIO-AVAILABILITY OF IRON FROM IRON FORTIFIED COW MILK-OAT MILK BLENDED YOGHURT**

DHAVALAGI PALLAVI<sup>1\*</sup>, JAYASHRI P. HIREMATH<sup>1</sup>, MADHUSUDAN.N.M.<sup>2</sup>

**AUTHOR DETAILS**

Received: 12<sup>th</sup> Aug 2016

Revised: 25<sup>th</sup> Sept 2016

Accepted: 30<sup>th</sup> Sept 2016

**Author details:**

<sup>1</sup>Department of Dairy Chemistry,

<sup>2</sup>Department of Dairy Microbiology,  
Dairy Science College, Hebbal,  
Bengaluru-56

**Corresponding author email:**

**ypallavi@gmail.com**

**ABSTRACT**

Yoghurt is a dairy product obtained by fermentation of milk using starter culture *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*. Application of dietary fibre and iron salt fortification in the manufacture of yoghurt enhances its nutritive and therapeutic value. Oat (*Avena sativa*) is a cereal that contains soluble fibre  $\beta$  – glucan renders several health benefits. In the present investigation, an attempt was made to develop iron salt fortified, cow milk and oat milk blended yoghurt and *in-vitro* bioavailability of the iron from the yoghurt was explored. The optimization of oat milk tried at different levels and was accepted at 20 % level and fortification of cow milk - oat milk blended yoghurt with ferrous sulphate fortified at different levels and was accepted at 10mg. Experimental yoghurt samples tested for sensory, chemical parameters, microbiological test and *in-vitro* bio-availability. The yoghurt samples showed (12%) of iron was maximum bio-availability from yoghurt fortified with ferrous sulphate followed by control and cow milk-oat milk blended yoghurt showed 10.2% and 8.52% respectively. The bio-availability of iron was reduced due to interfere of dietary fibre in yoghurt.

**Key words:** oat milk, yoghurt, ferrous sulphate and *In-vitro* bioavailability.

**INTRODUCTION**

Fermented foods are of great significance since they provide and preserve vast quantities of nutritious foods in a wide diversity of flavor, aroma and texture, which enrich the human diet [1]. Yogurt is a fermented dairy product obtained by lactic acid fermentation of milk by the action of yogurt starter bacteria, and is a popular product throughout the world. The highest production or consumption of yogurt is in Mediterranean, Asian countries and in central Europe. During recent years non-dairy milk types, such as soymilk, coconut milk, almond milk, mill milk, rice milk and oat milk, have been an increased demand from consumers due to their high functional properties. The cereal and grain milks aqueous extracts also do not contain cholesterol or lactose; hence, these milk types are preferred by health-

conscious people and lactose intolerant. Oats contain the best amino acid composition profile among all the cereal grains in addition to overall high protein content. Oat protein is uniquely different from other cereals. The higher level of lysine in the globulin fraction than in the glutelin and prolamin fractions counteracts the better nutritious value of oats [2]. Oat milk has recently attracted its research and commercial attention mainly due to its high nutritional value. Oat milk is free from lactose and is a good source of antioxidant vitamin E, phytic acid, phenolic acid and avenanthramides and soluble fibre beta-glucan. Yogurt is an excellent source of calcium and protein but, as is typical of all dairy products, contains very little iron [3]. Fortification of yogurt with iron would help to meet this nutritional need. An advantage of using dairy foods as the vehicle for supplementing the diet with iron is that people who consume diets of low iron

density typically consume more dairy products; those with diets high in iron consume the fewest dairy products. Furthermore, iron-fortified dairy foods have a relatively high iron bioavailability<sup>[4]</sup>. Variation in the bio-availability of iron (Fe) occurs because of interactions of food components in the gastro-intestinal micro environment. Bio-availability is preferably determined by in-vivo studies, but these are expensive, time consuming. As an alternative, in-vivo methods was used to predict bio-availability of nutrients from foodstuffs, which are useful for analysis and understand better about factors such as presence of inhibitors, chelating agents or certain enzymes that influence mineral uptake. Hence, the present study was proposed to know the effect of iron bioavailability from oat milk cow milk blended iron salt fortified yoghurt by an *In-vitro* method.

## MATERIALS AND METHODS

**Materials:** Cow milk was obtained from Student Experimental Dairy Plant, of Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), of Hebbal, Bengaluru and standardized to 4% fat and 8.5% SNF. Yoghurt cultures *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* in the form of freeze dried direct Vat set (FD-DVS) was obtained from Chr. Hansehs Laboratories, Copenhagen, Denmark were used in the study at the ratio of 1:1. Oat groats were procured from Sattvic foods, Goa, India. Ferrous sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ), pepsin enzyme (hog pancreas) and bile salt was obtained from Sigma-Aldrich chemicals company, Spain. Dialysis membrane with a molecular weight cut off of 10 KDa was obtained from HIMEDIA, India.

Study design: Analytical study

Study location: Department of Chemistry

Material: Oat milk and Yoghurt

### Preparation samples:

**Oat milk preparation:** The procedure of Patel and Ghosh., (2015)<sup>[5]</sup> was followed for preparation of oat milk with suitable modifications. Oat groats were cleaned and soaked in warm water overnight. The soaked oat groat was blended at the ratio of 1:4 oat to water and filtered through muslin cloth. The extract obtained was oat milk was heated to 60°C for 5min and cooled to room temperature.

**Yoghurt Preparation:** Yoghurt was prepared using the procedure followed by Lee and Lucey (2010)<sup>[6]</sup> adopted

with slight modifications in fat per cent. Standardized cow milk of 4% milk fat and 8.5% MSNF was heated to 95°C/5 min, then the milk was divided into 3 portions. The first portion was not blended with oat milk and not fortified with iron. The second portion was the cow milk oat milk blend in the ratio of 80:20 and not fortified with iron and third portion was cow milk oat milk blend in the ratio of 80:20 and fortified with 10mg of iron/kg milk. The milk was cooled to 42°C, inoculated with yoghurt culture at the level of 2 per cent and filled into 100 ml plastic cups and incubated at 42°C until firm curd was formed. The resultant yoghurt samples were analysed chemically, microbiologically, organoleptically and tested for *In-vitro* bioavailability of iron for fresh samples.

**Method of analysis:** Fat, protein, total solids, moisture and total ash was determined as per ISI: SP18 part XI (1981)<sup>[7]</sup>. The total fibre was determined by AOAC method (1980)<sup>[8]</sup>. The iron content was estimated by employing Atomic absorption spectrophotometer. Elliker's agar, was used for viable counts of yogurt cultures with incubation temperature of 37°C/24 to 48h at anaerobic condition. The violet red bile agar (VRBA) for coliform plates incubation at 37°C/18-24h and for yeast & mold malt extract agar was used incubated at 30°C/3-5days. Counts were taken after incubation and expressed the results as colony forming unit/g<sup>[9]</sup>.

**Sensory Evaluation:** Organoleptic properties of yoghurt samples were evaluated according to 9 point Hedonic scale<sup>[10]</sup>. Yoghurt was examined for colour and appearance, flavour, sourness, body and texture and over all acceptability.

***In-vitro* bioavailability of iron:** Dialysis method of Bosscher *et al.*, (2001)<sup>[11]</sup> was employed to determine the bio-availability of minerals.

The prime steps involved in this method are as follows.

### Intraluminal Digestion Phase

5 gm of sample was mixed with 40 ml of water in a 250ml conical flask. The pH was adjusted to 2.0 by adding 6M HCl. The pH was checked after 15min and if necessary readjusted. Freshly prepared 16% pepsin solution (1.5ml) was added and the sample was made upto 50 ml with distilled water. After mixing, the sample was incubated at 37°C in a shaking water bath at 1200 strokes/min for two hours, the gastric digest were stored in deep freeze for 90min.

**Determination of Total Titratable Acidity**

Titratable acidity was measured by taking a homogeneous pepsin digest (10ml) at 20±1°C and 2.5ml of freshly prepared 3:7 pancreatin bile mixture was added. The pH was adjusted to 7.5 with 0.5M NaOH. After an equilibrium period time of 30min, the pH was checked and readjusted to original pH if necessary. The number of equivalent of 0.5M NaOH required to titrate the amount of gastric digest to pH 7.5 was calculated.

**Pancreatin Digestion**

10 g of homogenized pepsin digest was weighed into wide necked conical flask, which was placed into water bath at 37°C for 5min. Segments of dialysis tubing (MWCO 10-12KDa) containing 25g of water and sodium bicarbonate being equivalents to the measured titratable acidity was placed into a wide necked conical flask were added to pepsin digest. Then seal the flask with aluminium foil and incubated in the shaking water bath at 37°C with continuous agitation (1200 strokes/min) until pH was about 5 (approximately 30min). Afterwards 2.5g of pancreatin bile mixture was added to digest, the digest was incubated in a shaking water bath for another 2 h at 37° C. At the end of incubation period the pH was measured. The dialysis bags were rinsed with water, the volume of dialysate was noted down. Iron content in the dialysate was estimated by means of Atomic Absorption Spectrophotometer (AAS).

**Calculation**

The availability of iron was calculated from the amount of element that passed through dialysis membrane related to the total element content of the original food sample.

$$\text{Availability (\%)} = \left( \frac{D-B}{W \times A} \right) \times 100$$

Where D = The total content of element in the dialysate (in mg)

B = The total amount of micronutrient (mg) in blank dialysate after digestion.

W = Weight of food sample for intestinal stage

A = Concentration of element in food sample

**Statistical analysis:** All measurements were done in the triplicates and analyzed using one way ANOVA using R software (R. version 3.1.3 (2015-03-09)).

**RESULTS**

**Table 1. Sensory attributes of yoghurt samples**

Yoghurt	Sensory attributes				
	Colour and appearance	Body and texture	Flavour	Sourness	Overall acceptability
C1	8.23 <sup>a</sup>	8.38 <sup>a</sup>	8.41 <sup>a</sup>	8.58 <sup>a</sup>	8.65 <sup>a</sup>
T <sub>1</sub>	8.13 <sup>a</sup>	8.40 <sup>a</sup>	8.26 <sup>a</sup>	8.32 <sup>b</sup>	8.40 <sup>b</sup>
T <sub>2</sub>	8.08 <sup>a</sup>	8.06 <sup>b</sup>	8.16 <sup>a</sup>	8.40 <sup>b</sup>	8.29 <sup>b</sup>
CD (P ≤ 0.05)	NS	0.21	NS	0.23	0.23

\*C1: cow milk yoghurt, T1: cow milk oat milk blended yoghurt, T2: cow milk oat milk blended yoghurt iron fortified yoghurt.

\*\*Similar superscripts indicate non-significant at the corresponding critical difference.

CD: Critical difference

NS: Non significant

**Table 2. Physico-chemical parameters of yoghurt samples**

Constituents (%) Yoghurt	Moisture	Protein	Fat	Total solids	Ash	Fibre
C1	85.20 <sup>a</sup>	3.50 <sup>a</sup>	4.00 <sub>a</sub>	14.70 <sub>a</sub>	0.70 <sub>a</sub>	-
T1	85.46 <sup>a</sup>	3.52 <sup>a</sup>	4.10 <sub>b</sub>	14.58 <sub>b</sub>	0.76 <sub>b</sub>	0.01 <sub>a</sub>
T2	85.45 <sup>a</sup>	3.51 <sup>a</sup>	4.10 <sub>b</sub>	14.62 <sub>b</sub>	0.76 <sub>b</sub>	0.01 <sub>a</sub>
CD (P ≤ 0.05)	NS	NS	0.08	0.07	0.01	NS

\*C1: cow milk yoghurt, T1: cow milk oat milk blended yoghurt, T2: cow milk oat milk blended yoghurt iron fortified yoghurt.

\*\*Similar superscripts indicate non-significant at the corresponding critical difference.

CD: Critical difference

NS: Non significant

**Table 3. Microbiological parameters of yoghurt samples**

Yoghurt samples	Microbial counts		
	C1	T1	T2
Total viable count (log <sub>10</sub> cfu/g)	8.13	8.20	7.93
Coliform count/gm	Nil	Nil	Nil
Yeast and mold count/gm	Nil	Nil	Nil

\*C1: cow milk yoghurt, T1: cow milk oat milk blended yoghurt, T2: cow milk oat milk blended yoghurt iron fortified yoghurt.

**Table 4. In-vitro bio-availability of iron from yoghurt samples**

Yoghurt samples	Total iron (mg/100g)	Bio-availability (%)
C1	0.10 <sup>a</sup>	10.20 <sup>a</sup>
T1	0.12 <sup>a</sup>	8.52 <sup>b</sup>
T2	1.04 <sup>b</sup>	12.00 <sup>c</sup>
CD (P ≤0.05)	0.08	0.10

\*C1: cow milk yoghurt, T1: cow milk oat milk blended yoghurt, T2: cow milk oat milk blended yoghurt iron fortified yoghurt.

\*\*Similar superscripts indicate non-significant at the corresponding critical difference.  
CD: Critical difference

## DISCUSSION

**Sensory attributes:** The colour and appearance scores were maximum for the control and lower for the optimized fortified yoghurt are shown in Table 1. The colour and appearance score for the cow milk-oat milk blended yoghurt fortified was higher than the yoghurt fortified with ferrous sulphate which may be attributed to the slight colour changes observed in the colour of

yoghurt. The maximum score was awarded to the cow milk-oat milk blended yoghurt. The better body and texture of the cow milk-oat milk yoghurt may be ascribed to the functional properties imparted by oat milk viz., water binding capacity whereas the cow milk-oat milk blended iron fortified yoghurt secured lower score as slight syneresis was observed. Similar finding was reported by Ramanathan and Sivakumar (2013) [12] in sweetened probiotic dahi with different levels of oats powder the increased scores in body and texture up to 2 per cent of oats powder addition. The decrease in the flavour in the cow milk-oat milk blended yoghurt (T1) may be ascribed due to lower production of flavour compounds by starter cultures as the cow milk availability was lowered. The control showed highest scores for sourness compared to cow milk-oat milk blended yoghurt and ferrous sulphate fortified yoghurt. The reason may be attributed due to the effect of fortificant. The maximum overall acceptability scores were awarded to the oat milk blended yoghurt, due to its better score for flavor and body and texture. Control and T2 samples had overall acceptability scores in the same range from like very much (8.00) to like extremely (9.00) and statistically non-significant. However, there was a significance between control and T1 and T2.

**Proximate composition:** Data presented in Table 2 show that the fat content of the cow milk-oat milk blended yoghurts samples with and without iron salts was higher than the control yoghurt (C1). The values were significantly different from the control which could be attributed to the contribution from oat milk. The cow milk-oat milk blended yoghurt (T1) and cow milk-oat milk blended yoghurt with iron (T2) was found to contain slightly higher levels of protein, due to higher percentages of protein in oat milk. The control sample showed higher acidity and lesser acidity of the formulated yoghurt samples may be attributed due to the effect of low lactose content, low caseins content, oat milk proteins and iron salt in the optimized product. The moisture content in all the yoghurt samples was more or less the same. The total solid content of the optimized yoghurt was less when compared to control. The decreased level of total solids in the cow milk-oat milk blended yoghurt is due to addition of oat milk.

**Microbiological attributes:** According to the data presented in Table 3 the total viable count on the for

control, cow milk-oat milk blended yoghurt (T1), cow milk-oat milk blended ferrous sulphate fortified yoghurt (T2) were 8.13, 8.20 and 7.93 log<sub>10</sub> cfu/gm respectively. The total viable bacterial count was higher in T1 than in control (C1). This may be due to the prebiotic effect of traces of fibre contributed by oat milk whereas the viable bacterial counts were relatively lower in case of yoghurt fortified with ferrous sulphate (T2) might be due to the effect of iron salt. As per the FSSAI recommendation the coliforms and yeast and mold should be not more than 10/gm in yoghurt. The coliform count and yeast and mold count shown in Table 2 for all the three yoghurt samples was nil in fresh yoghurt.

**Bioavailability of iron:** As shown in Table 4 *In-vitro* studies on bio-availability of iron indicated higher bioavailability from fortified yoghurt with ferrous sulphate i.e., 12.00%. The bio-availability from unfortified plain cow milk yoghurt and cow milk oat milk blended yoghurt were observed to be only 10.20 % and 8.52% respectively. The results were statistically significant. The observed decrease in the absorbable iron fraction might be due to incorporation of oat milk as it contains the soluble dietary fibre beta-glucan which interfere with iron by combining with it. The present investigation's results are in conformity with findings of Staffolo *et al.*, (2011) <sup>[13]</sup>, who reported that the bioavailability varies according to the type of dietary fibre.

## CONCLUSION

From the present study, it may be concluded that a choice of cow milk-oat milk blended yoghurt and fortification with iron cannot be done together simultaneously. A choice of either oat milk or iron salt in the yoghurt is preferable as the oat milk impaired bio-availability of iron from yoghurt.

## REFERENCES

1. S. Sarkar, A.K. Misra. Yogurt: nutritional and therapeutic significance, Indian J. Microbial. 2002, 42:275-287.
2. M. Salehifar, M. Shahedi. Effects of oat flour on dough rheology, texture and organoleptic properties of taftoon bread, J. Agric. Sci. Technol. 2007, 9:227-234.
3. Blanc, B. Biochemical aspects of human milk comparison with bovine milk. World Rev. Nutr. Diet. 1981, 36:1.
4. Woestyne, M. V., B. Bruyneel, M. Mergeay, and W. Verstraete. The Fe<sup>2+</sup> chelator proferrerosamine A is essential for the siderophore-mediated uptake of iron by *Pseudomonas roseus fluorescens*. Appl. Environ. Microbiol. 1991. 57:949.
5. Patel, D.H. and Ghosh, B.C. Oats-beneficial attributes and application in foods. Indian Food Industry Mag. 2015, vol.34
6. Lee, W.J .and Lucey, J.A. Formation and physical properties of yogurt. J. Anim.Sci., 2010, 23(9):1127-1136
7. ISI Hand book of food analysis, Part XI Dairy products. Indian Standards Institute. 1981 Manak Bhavan, New Delhi, pp 167-173.
8. AOAC. Official methods of analysis 13th Ed. 1980. Association of Official Agricultural Chemist, Washington, D.C., USA.
9. Harrigan, W.F. Laboratory methods in food and dairy microbiology, Academic press, Inc. (London) Ltd., U.K. 1998.
10. Jun Lim, Hedonic scaling: A review of methods and theory, Food quality and preference 2011, 22:737-747.
11. Bosscher Douwina., Van Caillie-Bertr., Micheline., Robberecht., Harry., Van Dyck., Kristien., Van Cauwenbergh., Rudy., Deelstra and Hendrik. In-vitro availability of calcium, iron, and zinc from first-age infant formulae and human Milk. J. Pediatr. Gastroenterol. Nutr., 2001, 32: 54-58.
12. Ramanathan, A. and Sivakumar, K. Evaluation of fibre enriched and vitamin C fortified sweetened probiotic dahi. Probiotics in sustainable food production: current status and future projects-Probiotic foods. 2013.
13. Staffolo Dello Marina., Bevilacqua, E. Alicia., Rodriguez Susanmaria. and Albertengo L. Chitosan. Interaction with iron from yoghurt using an in-vitro digestion model: comparative study with plant dietary fibers. Int.J.Molecular. Sci., 2011, 12:4647-4660.