

# Evaluation of Storage Capacity of Iron Fortified Yogurt by Physico-chemical, Chemical and Microbiological Analysis

Debasmita De<sup>1</sup>, Dr. Binata Nayak<sup>2</sup>

<sup>1</sup> Junior Research Fellow, Department of Home Science (Food & Nutrition Division) University of Calcutta, Kolkata, West Bengal, India

<sup>2</sup> Assistant Professor, Department of Home Science (Food & Nutrition Division) University of Calcutta, Kolkata-700027, West Bengal, India

**Abstract**—Yogurt has gained widespread consumer acceptance. It is excellent source of calcium and protein and other nutrients but it contains very little iron. In this study yogurt was fortified with ammonium ferrous sulfate in three different concentrations (20mg, 30mg, 40mg/kg iron). Yogurt samples were analyzed physico-chemically, chemically and microbiologically at 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> day of storage. Physicochemical and chemical result shows that there was significant difference between storage period and different sample concentration. Iron in the fortified samples had no significant effect in *Lactobacillus* count. The results suggest possibility of making good quality yogurt by fortifying milk with ammonium ferrous sulfate.

**Keywords**—Ammonium ferrous sulphate, Chemical, fortification, Microbiological, Physicochemical, Yogurt.

## I. INTRODUCTION

Anaemia is a most common world-wide problem in the young children, pregnant woman and adolescent girl. Nutritional anaemia may be defined as the condition that results from the inability of the erythropoietic tissues to maintain a normal haemoglobin concentration on account of inadequate supply of one or more essential nutrients leading to reduction in the total circulating haemoglobin.[1] Most of the anaemias are due to inadequate supply of nutrients like iron, folic acid and vitamin B12, proteins, amino acids, vitamins A, C, and other vitamins of B-complex group i.e., niacin and pantothenic acid are also involved in the maintenance of haemoglobin level.[2]

Globally, anaemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in preschool-age children (47.4%), and the lowest prevalence is in men (12.7%). However, the population group with the greatest number of individuals affected is pregnant women (41.8%) [3]. In women, anaemia may become the underlying cause of maternal mortality and perinatal mortality. Nearly 50 per cent of

women of reproductive age and 26 per cent of men in the age group of 15-59 years are anaemic[4].

To reduce the prevalence rate of anaemia three types of measures are taken: 1. Dietary improvement, 2. Supplementation, 3. Food fortification.[1],[5],[6] The iron found in food can be highly bioavailable as in the case with heme iron which is found in red meat. The iron present in other products of vegetable origin contain non-heme iron has disadvantage of interacting with substance in food that inhibits absorption such as tannin and phytates[7]. The best way to prevent problems associated with iron deficiency is through iron fortification of food for whole population or certain group [8]. Yogurt has gained widespread consumer acceptance. It is excellent source of calcium and protein, but it contains very little iron. Therefore dairy products are good for iron fortification because they have high nutritive value reach target population and are widely consumed[9],[10],[11]. The ideal iron compound used as fortificants should supply high bioavailability iron, it should not affect the nutritional value or sensory properties of food [12],[13],[14],[15].

The purpose of this study is to prepare iron-fortified yogurt with ammonium ferrous sulfate at three different concentration (20mg, 30mg, 40mg/kg milk) as it covers respectively 9.52%, 14.28% and 19.04% of RDA of iron of an adult woman. And yogurt samples were analyzed chemically and microbiologically during 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> day of storage.

## II. MATERIALS & METHODS

The study was designed to prepare Iron fortified yogurt and physicochemical, chemical, microbial analysis of iron fortified yogurt at 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> day of storage period. Methodologies adopted for this analysis:

1. Preparation of iron fortified yogurt.[6]
2. Physicochemical analysis of iron fortified yogurt
  - i) Whey separation by centrifugation method. [16]
  - ii) Volume of supernatant by syneresis index. [16]

- iii) Determination of Total TitratableAcidity .[17]
  - iv) Moisture content determination. [18]
  - v) Total solid content determination. [18]
3. Chemical analysis of iron fortified yogurt.

- i) Determination of protein by Lowry method. [19]
  - ii) Determination of iron by Wong’s method. [20]
4. Microbial analysis of iron fortified yogurt.
- i) Enumeration of Lactobacillus count. [21]

**Preparation of Iron fortified Yogurt:** Locally available AmulTaja toned homogenized pasteurized milk was taken. Milk was fortified with ammonium ferrous sulfate. The milk was divided into four portions. The first portion was not fortified with iron and regarded as control. The rest three portions were fortified with ammonium ferrous sulfate in different concentration respectively 20mg, 30mg, 40mg iron/kg milk. Then milk was inoculated with yogurt culture and filled into plastic cups, covered and kept at room temperature until a firm curd was formed (approximately 6-7 hours). The resultant yogurt was kept in a refrigerator for 5 days at 4°C.[6]

**Statistical analysis:** This was done by Two way Analysis of Variance (first factor storage period, second factor sample concentration).

### III. RESULT & DISCUSSION

#### 3.1 Physicochemical analysis

##### 3.1.1 Volume of whey

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	0.592 ±0.001	0.672 ±0.009	0.866 ±0.02	Between columns=34.85	0.00084
Yogurt (20mg)	0.694 ±0.009	0.86 ±0.06	0.91 ±0.03	Between rows=404.10	0.0005
Yogurt (30mg)	1.293 ±0.05	1.399 ±0.02	1.496 ±0.02		
Yogurt (40mg)	1.475 ±0.01	1.576 ±0.04	1.636 ±0.01		

**Inference:** Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted.

##### 3.1.2 Volume of supernatant by syneresis index (%)

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	11.85 ±0.03	13.44 ±0.19	17.32 ±0.48	Between columns=38.74	0.00075
Yogurt (20mg)	13.88 ±0.19	17.2 ±1.2	18.2 ±0.66	Between rows=430.30	0.0005

Yogurt (30mg)	25.86 ±1.03	27.98 ±0.48	29.92 ±0.53		
Yogurt (40mg)	29.2 ±0.5	31.52 ±0.81	32.72 ±0.3		

**Inference:** Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

##### 3.1.3 Total Titratable Acidity (%):

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	0.27 ±0.005	0.29 ±0.005	0.32 ±0.01	Between columns=19.90	0.0021
Yogurt (20mg)	0.27 ±0	0.28 ±0.005	0.29 ±0.005	Between rows=6.18	0.03538
Yogurt (30mg)	0.27 ±0.005	0.30 ±0.005	0.31 ±0.005		
Yogurt (40mg)	0.28 ±0.005	0.32 ±0.01	0.34 ±0.01		

**Inference:** Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

##### 3.1.4 Moisture(%):

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	86 ±0.7	87.2 ±0.5	88.8 ±1	Between columns=27.06	0.00119
Yogurt (20mg)	87.4 ±0.5	88.8 ±0.27	89.14 ±0.91	Between rows=9.08	0.0158
Yogurt (30mg)	87.6 ±0.9	88.4 ±0.7	89.2 ±0.63		
Yogurt (40mg)	88 ±0.7	89 ±1.7	89.4 ±0.77		

**Inference:** Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

##### 3.1.5 Total solid(%):

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	14 ±0.7	12.8 ±0.5	11.2 ±1	Between columns=30.09	0.00101
Yogurt (20mg)	12.6 ±0.5	11.2 ±0.27	10.86 ±0.91	Between rows=9.04	0.01593
Yogurt (30mg)	12.4 ±0.9	11.6 ±0.7	10.8 ±0.63		

Yogurt (40mg)	12 ±0.7	11 ±1.7	10.6 ±0.77		
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**Inference:** Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

### 3.2 Chemical analysis:

#### 3.2.1 Protein (mg/ml):

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	31 ±0.5	31.3 ±0.9	31.4 ±1.2	Between columns=26.99	0.00119
Yogurt (20mg)	31.5 ±0.5	32 ±1	32.3 ±0.7	Between rows=80.98	0.00054
Yogurt (30mg)	32 ±2	32.4 ±0.6	32.5 ±0.5		
Yogurt (40mg)	32.2 ±0.11	32.44 ±0.55	32.56 ±1.05		

**Inference:** Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

#### 3.2.2 Iron (µg/ml)

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	4 ±0.5	3.5 ±0.2	2.5 ±0.4	Between columns=35.80	0.00081
Yogurt (20mg)	21.06 ±0.40	20.03 ±1.15	19.5 ±0.8	Between rows=13149.72	0.0005
Yogurt (30mg)	31.5 ±0.8	31 ±0.5	30.5 ±0.5		
Yogurt (40mg)	43.5 ±0.5	42.2 ±0.75	41.53 ±0.95		

**Inference:** Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

### 3.3 Microbial analysis

#### 3.3.1 Lactobacillus count (cfu/ml) 10<sup>10</sup>

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	10.2 ±0.3	9.6 ±0.2	9.9 ±0.1	Between columns=1.13	0.40785
Yogurt (20mg)	11.8 ±0.8	11.03 ±0.55	11.3 ±0.3	Between rows=4.44	0.06589
Yogurt (30mg)	9 ±0.5	9.5 ±0.5	11.03 ±1.05		
Yogurt (40mg)	11 ±0.1	10.3 ±0.5	10.7 ±0.3		

**Inference:** Two Way ANOVA shows that there was a non-significant difference between columns and rows. So null hypothesis is accepted.

## IV. CONCLUSION

Yogurt is a most important health beneficial nutritious probiotic. It is a product of the lactic acid fermentation of milk. Result shows that during storage period volume of syneresis increased in all samples but this increase is significant in non-fortified sample. There was a steady increase in Total titratable acidity during storage period. The pH was decreasing due to accumulation of lactic acid as a bacterial culture was breaking down lactose in order to obtain energy. This observation is in agreement with the previous study by Nkhata et al[22]. The metabolic enzymatic activity of the yogurt starter culture could be the reason for increases in acidity which could be responsible for decreasing lactobacillus spp. count although statistical analysis shows there was nonsignificant difference in lactobacillus count during storage period and sample concentration. Statistical analysis of moisture, total solid, iron and protein shows that there was a significant difference between sample concentration and storage period. Present study shows all fortified yogurt samples were nutritionally rich and acceptable, suggesting that yogurt is a suitable vehicle for iron fortification.

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