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RESEARCH ARTICLE

EVALUATION OF ORGANOLEPTIC &IN-VITRO BIOAVAILABILITY PROPERTIES OF IRON FORTIFIED YOGURT

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Abstract

Yogurt is a fermented dairy product formed by the action of Lactobacillus spp. with the help of lactic acid fermentation procedure.In India major cause of anaemia is dietary iron deficiency.Iron deficiency causes poor cognitive performance, behavioural abnormalities, poor physical growth of children, disturbed immune function, lack of physical activity and poor work performance in all age groups. Worldwide dairy foods are most popular nutritious product so dairy foods may be used as a vehicle of food fortification. The iron compound which are used as a food fortificant should contain highly bioavailable iron and the physical chemical characteristics of the fortified food product should be similar to non-fortified product. 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. Sensory evaluation and In-vitro bioavailability study of yogurt samples were analyzed at 1st, 4th, 7th day of storage. Statistical analysis of the study shows ferrous sulphate and iron-casein complex fortified yogurt enhances the iron absorption compared to ammonium ferrous sulphate fortified yogurt.

Introduction and Review of literature:-

Micronutrients are essential vitamins and minerals which required on a daily basis to ensure good health and to enable the body to fight disease. They are referred to as micronutrients because individual need them in small quantities for proper growth and development.

India has a high prevalent of micronutrient deficiency disease such as anaemia, vitamin A deficiency and iodine deficiency in all age groups and socioeconomic conditions, but consequences are severe when it affects children below 24 months of age since they are largely irreversible.[1]

Indian diet is typically cereal based, intake of foods rich in micronutrient is far lesser than the Recommended Dietary Allowance in all age groups. Dietary diversification, fortification, supplementation and bio-fortification along with other measures like infection control, water and sanitation etc. are strategies to tackle micronutrient malnutrition. [2]

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Food fortification or enrichment is the process of adding micronutrients to the food [3]. Sometimes it is a purely commercial choice to provide extra nutrients in a food where other times it is a public health policy which aims to reduce the number of people with dietary deficiencies within the population [4] [5]. Sometime staple foods of region can lack particular nutrients due to the soil of the region or from inherent inadequacy of a normal diet. Addition of micronutrients to the staple food can prevent large scale deficiency diseases [6].

Iron is a micronutrient in the human body that need our body's many functions. Iron is needed for growth and development and to make both hemoglobin and myoglobin. Hemoglobin is a protein found in red blood cells that carries oxygen from our lungs to the rest of our body, and myoglobin is a protein that brings oxygen to our muscles [7].

Iron is a micronutrient in the human body that need our body's many functions. Iron is needed for growth and development and to make both hemoglobin and myoglobin. Hemoglobin is a protein found in red blood cells that carries oxygen from our lungs to the rest of our body, and myoglobin is a protein that brings oxygen to our muscles [7].

The bioavailability of iron from fortificants is dependent not only on the solubility of the fortificant, but also on the composition of the diet, in particular, on the amounts of inhibitors of iron absorption in the diet, for example iron-binding phytates and certain phenolic compounds. The addition of ascorbic acid (vitamin C) or sodium ethylene diamine tetra acetic acid (sodium EDTA or Na2EDTA) and the removal of phytates, all of which reduce the effect of the iron inhibitors, can be effective ways of increasing the total amount of iron absorbed from iron-fortified foods [8].

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. Yogurt comes from milk, it is reach in animal protein, plus several other nutrients are also found in dairy foods, like calcium, vitamin B-2, vitamin B-12, potassium, and magnesium. Yogurt act as probiotic, as it contains strains of bacterial species which act as naturally occurring gut microflora. Some evidence shows that some strains of probiotics can help to boost the immune system and promote a healthy digestive tract [9].

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 daily basis. In-vitro bioavailability study of yogurt samples was analyzed on 1st, 4th, 7th day of storage.

Material and Methods:-

Preparation of Iron fortified Yogurt:

Locally available 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 [10]

Organoleptic study:

Organoleptic study of 30 combination of yogurt sample at 1^{st}, 4^{th} and 7^{th} day of storage period by Nine-point hedonic scale with 5 sensory characteristics (colour, smell, consistency, taste, overall quality) on 50 subject 18-25 years of age. [11]

Invitro-bioavailability study:

Total iron of yogurt sample was estimated by the wong's method. [12]

Ionizable iron extraction from sample:

At pH 1.35:

2gm of sample was taken in a 100ml conical flask and 25 ml of Pepsin-HCl solution was mixed with it (0.5% pepsin in 0.1N HCl). The pH of the sample solution was adjusted to 1.35 with distilled HCl. The sample was incubated for

90minute at 37°c in a metabolic shaker water bath. At the end of this procedure the contents of conical flask were centrifuged at 3000rpm for 45 minutes. Then the supernatant was filtered by whatman no.44 filter paper. Aliquots of the filtrate was used for determination of ionizable iron. [4]

At pH 7.5:
Another aliquot of the sample which was available from sample and Pepsin –HCl mixed solution, pH was adjusted to 7.5 with NaOH. The sample was incubated for 90minute at 37°c in a metabolic shaker water bath. At the end of this the procedure the contents of the flask were centrifuged at 10000-15000 rpm for 45 minutes. Then the supernatant was filtered by whatman no.44 filter paper. Aliquots of the filtrate was used for determination of ionizable iron. [4]

Estimation of ionizable iron and prediction of iron bioavailability by prediction equation was reported by Narasinga Rao& Prabhavathi [13]. This equation correlated the physiological availability of iron in humans with the ionizable iron at pH 7.5

Based on this correlation a prediction equation on iron absorption was formulated.

The prediction equation is: Y=0.4827+0.470X
Where, Y-is the % of iron absorption in adult
X-is the % of ionizable iron at pH 7.5

Statistical analysis:
Invitro Bioavailability study of Yogurt fortified with different iron salts and in different concentration at 1^{st} 4^{th} and 7^{th} day of storage period was statistically analyzed by One-way ANOVA method. Comparisons among Fortified yogurt samples with control at individual day variation was analyzed by One-way ANOVA method. Differences were considered to be significant when p value is < 0.05. Data was analyzed by using SPSS package.

For organoleptic study statistical analysis was done by chi- square method and Differences were considered to be significant when p value is < 0.05.

Results and Discussion:-

Organoleptically Study

Colour

Table no 1:-Mean,Standard Deviation and Chi-square value of non-fortified and iron fortified yogurt samples at three different storage day at the temperature of 4(±2°c).

Treatment	1st	4th	7th
NF	8.22±0.74	8.08±0.77	8.26±0.59
AFS 20	8.42±0.63	8.29±0.77	8.27±0.68
AFS 30	8.16±0.85	7.93±1.13	8.06±0.72
AFS 40	7.98±0.96	8.11±0.89	8.01±1.10
FS 20	8.05±0.75	7.94±1.24	7.87±1.43
FS 30	8.07±0.54	8.31±0.95	8.05±1.00
FS 40	8.11±0.75	8.28±0.83	7.86±1.02
IC 20	8.16±0.69	8.04±1.43	8.05±1.24
IC 30	8.11±0.75	8.05±0.74	8.00±0.79
IC 40	8.38±0.59	7.98±1.06	8.12±0.76
Chi-Square	0.021	0.023	0.021
df	9	9	9
Asymp. Sig.	0.999	0.999	0.999

Smell

Table no 2:- Mean, Standard Deviation and Chi-square value of non-fortified and iron fortified yogurt samples at three different storage day at the temperature of 4(±2°c).

Treatment	1st	4th	7th
NF	7.29±1.18	7.18±1.48	7.24±1.39
AFS 20	7.42±1.06	7.09±1.31	7.08±1.43

AFS 30	7.44±1.14	7.02±1.18	7.12±1.26
AFS 40	7.13±1.62	7.19±1.46	7.22±1.69
FS 20	7.29±1.50	7.23±1.53	7.03±1.65
FS 30	7.05±1.21	7.11±1.35	7.26±1.62
FS 40	7.27±1.62	7.05±1.46	7.15±1.00
IC 20	7.47±1.40	7.21±1.55	7.21±1.50
IC 30	7.44±1.13	7.07±1.29	7.11±1.45
IC 40	7.71±0.91	7.13±1.50	7.04±1.26
Chi-Square	0.043	0.006	0.008
df	9	9	9
Asymp. Sig.	0.999	1	1

Consistency

Table no 3:-Mean,Standard Deviation and Chi-square value of non-fortified and iron fortified yogurt samples at three different storage day at the temperature of 4(±2°c).

Treatment	**1st**	**4th**	**7th**
NF	7.27±0.96	7.32±0.90	7.23±1.39
AFS 20	7.35±0.74	7.35±0.92	7.39±1.37
AFS 30	7.44±1.41	7.47±1.15	7.47±1.29
AFS 40	7.29±1.24	7.39±1.16	7.55±1.46
FS 20	7.78±1.04	7.65±1.14	7.28±1.46
FS 30	7.45±1.48	7.19±1.04	7.33±1.36
FS 40	7.41±1.09	7.27±1.25	7.72±1.23
IC 20	7.17±0.64	7.53±1.32	7.29±1.60
IC 30	7.23±1.10	7.36±0.83	7.25±1.19
IC 40	7.62±0.65	7.22±1.18	7.32±1.27
Chi-Square	0.041	0.024	0.029
df	9	9	9
Asymp. Sig.	0.999	0.999	0.999

Taste

Table no 4:-Mean,Standard Deviation and Chi-square value of non-fortified and iron fortified yogurt samples at three different storage day at the temperature of 4(±2°c).

Treatment	**1st**	**4th**	**7th**
NF	7.56±1.06	7.07±1.16	7.04±1.47
AFS 20	7.61±1.07	7.36±1.51	7.27±1.39
AFS 30	7.56±0.81	7.47±1.37	7.35±1.06
AFS 40	7.33±1.42	7.35±1.67	7.29±1.46
FS 20	7.07±0.97	7.58±1.84	7.41±1.55
FS 30	7.87±1.14	7.22±1.55	7.19±1.38
FS 40	7.39±1.21	7.09±1.49	7.22±1.40
IC 20	7.21±1.17	7.26±1.96	7.35±1.55
IC 30	7.34±1.41	7.52±1.26	7.21±1.67
IC 40	7.52±1.08	7.17±1.38	7.39±1.18
Chi-Square	0.062	0.038	0.015
df	9	9	9
Asymp. Sig.	0.999	0.999	0.999

Overall Quality

Table no 5:-Mean,Standard Deviation and Chi-square value of non-fortified and iron fortified yogurt samples at three different storage day at the temperature of 4(±2°c).

Treatment	**1st**	**4th**	**7th**
NF	7.60±0.73	7.47±0.79	7.72±0.97
AFS 20	7.82±0.76	7.61±0.90	7.67±0.99
AFS 30	7.86±0.81	7.42±0.97	7.68±1.02

AFS 40	7.76±1.12	7.48±0.99	7.55±1.12
FS 20	7.84±0.85	7.59±1.20	7.63±1.32
FS 30	7.58±0.75	7.65±1.03	7.51±1.15
FS 40	7.72±1.09	7.39±1.05	7.49±0.95
IC 20	7.67±0.70	7.42±1.05	7.51±1.21
IC 30	7.59±0.76	7.41±0.72	7.64±1.17
IC 40	7.65±0.64	7.57±0.97	7.55±0.94
Chi-Square	0.013	0.010	0.008
df	9	9	9
Asymp. Sig.	0.999	0.999	0.999

In-vitro bioavailability study

Table no 6:- Comparisons among Fortified yogurt samples with control at 1st, 4th and 7th day of storage period At pH 1.35.

Sample name	Storage day	Total iron content	Ionizable iron	% of total iron	F value	P value
NF	1st day	4 ±0.1	1.6 ±0.03	40 ±0.75	627.8795	0.0001
AFS 20		21.06 ±0.16	8.16 ±0.04	38.74 ±0.19		
AFS 30		31.5 ±0.15	12.37 ±0.08	39.26 ±0.25		
AFS 40		42.6 ±0.13	16.23 ±0.085	38.1 ±0.18		
FS 20		21 ±0.24	10.56 ±0.09	50.28 ±0.43		
FS 30		31.5 ±0.1	15.69 ±0.07	49.80 ±0.22		
FS 40		41.2 ±0.28	20.64 ±0.13	50.09 ±0.31		
IC 20		21.2 ±0.2	10.33 ±0.06	48.72 ±0.28		
IC 30		31.33 ±0.05774	15.66 ±0.15	49.99 ±0.48		
IC 40		41.5 ±0.1	20.65 ±0.016	49.75 ±0.38		
NF	4th day	3.8 ±0.19	1.5 ±0.09	39.54 ±2.37	143.5908	0.0001
AFS 20		20.6 ±0.3	7.94 ±0.05	38.55 ±0.24		
AFS 30		31.2 ±0.2	12.10 ±0.13	38.94 ±0.248		
AFS 40		42.2 ±0.1	16 ±0.21	37.91 ±0.50		
FS 20		20.9 ±0.09	10.41 ±0.04	49.82 ±0.19		
FS 30		31.5 ±0.2	15.51 ±0.09	49.24 ±0.285		
FS 40		40.96 ±0.05774	20.42 ±0.11	49.89 ±0.32		
IC 20		21.1 ±0.1	10.19 ±0.02	48.29 ±0.09		
IC 30		31.1 ±0.05	15.41 ±0.02	49.55 ±0.06		
IC 40		41.3 ±0.44	20.38 ±0.14	49.35 ±0.34		
NF	7th day	3.5 ±0.1	1.36 ±0.05	38.90 ±1.43	267.7102	0.0001
AFS 20		20.24 ±0.14	7.71 ±0.08	38.09 ±0.39		
AFS 30		30.9 ±0.7	11.89 ±0.15	38.48 ±0.48		
AFS 40		41.9 ±1.1	15.75 ±0.06	37.59 ±0.14		
FS 20		20.3 ±0.4	9.94 ±0.04	48.97 ±0.2		
FS 30		31 ±0.1	15.06 ±0.17	48.58 ±0.54		
FS 40		40.8 ±0.3	20.17 ±0.07	49.43 ±0.17		
IC 20		20.9 ±0.2	9.96 ±0.11	47.66 ±0.53		
IC 30		31 ±0.1	15.16 ±0.04	48.90 ±0.13		
IC 40		41.1 ±0.15	20.02 ±0.13	48.71 ±0.31		

From the P value of F test table we can concluded that there was significant difference among fortified yogurt samples with control at 1st, 4th and 7th day of storage period at pH 1.35.

Table no7:-Comparisons among Fortified yogurt samples with control at 1st, 4th and 7th day of storage period At pH 7.5.

Sample name	Storage day	Total iron content	Ionizable iron	% of total iron	F value	P value
NF	1st day	4 ±0.1	0.82 ±0.08	20.52 ±2.00	175.3014	0.0001
AFS 20		21.06 ±0.16	4.35 ±0.03	20.65 ±0.14		

AFS 30		31.5 ±0.15	6.60 ±0.07	20.95 ±0.22		
AFS 40		42.6 ±0.13	8.65 ±0.04	20.30 ±0.09		
FS 20		21 ±0.24	6.51 ±0.15	31 ±0.71		
FS 30		31.5 ±0.1	9.87 ±0.05	31.33 ±0.16		
FS 40		41.2 ±0.28	12.38 ±0.09	30.05 ±0.21		
IC 20		21.2 ±0.2	6.67 ±0.03	31.47 ±0.14		
IC 30		31.33 ±0.05774	9.69 ±0.07	30.95 ±0.22		
IC 40		41.5 ±0.1	12.44 ±0.09	29.97 ±0.22		
NF	4^{th} day	3.8 ±0.19	0.76 ±0.01	20.07 ±0.29	625.7547	0.0001
AFS 20		20.6 ±0.3	4.14 ±0.09	20.1 ±0.43		
AFS 30		31.2 ±0.2	6.43 ±0.18	20.60 ±0.57		
AFS 40		42.2 ±0.1	8.46 ±0.06	20.04 ±0.14		
FS 20		20.9 ±0.09	6.28 ±0.04	30.05 ±0.19		
FS 30		31.5 ±0.2	9.79 ±0.16	31.08 ±0.5		
FS 40		40.96 ±0.05774	12.16 ±0.14	29.69 ±0.34		
IC 20		21.1 ±0.1	6.35 ±0.06	30.1 ±0.28		
IC 30		31.1 ±0.05	9.44 ±0.12	30.35 ±0.38		
IC 40		41.3 ±0.44	12.24 ±0.04	29.63 ±0.10		
NF	7^{th} day	3.5 ±0.1	0.69 ±0.07	19.75 ±2.00	149.8394	0.0001
AFS 20		20.24 ±0.14	3.88 ±0.08	19.18 ±0.39		
AFS 30		30.9 ±0.7	6.22 ±0.09	20.12 ±0.29		
AFS 40		41.9 ±1.1	8.23 ±0.12	19.64 ±0.28		
FS 20		20.3 ±0.4	5.90 ±0.03	29.07 ±0.15		
FS 30		31 ±0.1	9.52 ±0.15	30.71 ±0.48		
FS 40		40.8 ±0.3	11.95 ±0.12	29.28 ±0.29		
IC 20		20.9 ±0.2	5.99 ±0.14	28.66 ±0.67		
IC 30		31 ±0.1	9.20 ±0.06	29.68 ±0.19		
IC 40		41.1 ±0.15	12.04 ±0.03	29.29 ±0.07		

From the P value of F test table we can concluded that there was significant difference among fortified yogurt samples with control at 1^{st}, 4^{th} and 7^{th} day of storage period at pH 7.5.

Table no 8:- Predicting equation result chart at 1^{st}, 4^{th} and 7^{th} day storage period.

Sample	**Predicting Bioavailability(%) at 1^{st} day storage period**	**Predicting Bioavailability(%) at 4^{th} day storage period**	**Predicting Bioavailability(%) at 4^{th} day storage period**
NF	10.14	9.92	9.77
AFS 20	10.20	9.94	9.51
AFS 30	10.34	10.17	9.95
AFS 40	10.03	9.91	9.72
FS 20	15.07	14.62	14.16
FS 30	15.60	15.11	14.93
FS 40	14.62	14.45	14.26
IC 20	15.29	14.65	13.97
IC 30	15.05	14.76	14.45
IC 40	14.58	14.42	14.26

The predicted bioavailability of iron fortified yogurt with Ferrous sulphate (20,30,40mg/kg milk) and Iron casein complex (at 20,30,40mg/kg milk) give higher value comparison with non-fortified & Ammonium Ferrous sulphate fortified sample.

From this studies it can be interpreted that yogurt fortified with ferrous sulfate & iron-casein complex enhances the iron absorption in humans.

Summary and Conclusion:-

Iron-fortified yogurt was prepared with three different iron fortificants Ammonium ferrous sulfate, Ferrous sulfate, Iron-casein complex, at different concentrations (20mg/kg milk, 30mg/kg milk, 40mg/kg of milk) which makes respectively 6.89%, 10.34% and 13.79% of RDA of iron of an adult Indian woman (According to RDA 2020).

Organoleptic study shows no significant difference in color, smell, consistency, taste and overall quality at the 1st, 4th and 7th day of the storage period in all samples compared to control.

In-vitro bioavailability study was done with iron-fortified and non-fortified yogurt at 1st, 4th and 7thday of storage period at pH 1.35 all the compositions showed statistically better result than non- fortified and ammonium ferrous sulphate fortified samples during throughout storage period. A similar result was obtained for in-vitro bioavailability study for pH 7.5 throughout the storage period. The predicted bioavailability of iron-fortified yogurt with Ferrous sulfate (20, 30, 40mg/kg milk) and Iron casein complex (at 20, 30, 40mg/kg milk) give a higher value compared with non-fortified & Ammonium Ferrous sulfate fortified sample. The ammonium ferrous sulfate fortified yogurt at pH 1.35 was more stable than other fortificant. Although ferrous sulfate and iron-casein complex were less stable during storage conditions, their ionizable iron percentage value was more than ammonium ferrous sulfate during store conditions.

NF, AFS 30, FS 30 was more stable than other yogurt samples at pH 7.5 during store condition. Ionizable iron percentage value was higher in ferrous sulfate and iron casein complex fortified yogurt than ammonium ferrous sulfate during store condition. So, this two fortificant gives a better result at pH 7.5.

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