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EFFECT OF FORTIFICATION ON TEXTURAL, MICROBIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF BREAD

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ABSTRACT

The effect of fortification with *Spinacia oleracea* L. on the physico-chemical, sensory value, textural properties and microbiological analysis of bread was carried out. Breads were made by substituting wheat flour with spinach powder (SP) at 0, 1, 2.5, 5% and Ferrous Sulphate powder (FSP) ($FeSO_4$. 7 H_2O) at 0.025, 0.05, and 0.10% levels. Addition of different levels of fortificants showed a marginal effect on the textural and physico-chemical properties. Significant increase in iron content was observed even with low levels of fortification. The hardness, chewiness and gumminess of bread samples increased during storage. Total plate count, yeast and mold count showed slight decreasing trend within treatments and an increasing trend during the storage from 0.14-1.45×10^2cfu/g and 1.4 to 2.7 x 10^1cfu/g respectively.

1. Introduction

Cereal based diet is primarily the basic diet in India besides other Asian countries. This type of diet has basically less bioavailability of micronutrients including deficiency of iron, vitamin A and anaemia. Iron deficiency anaemia (IDA) is a general nutritional disorder among the young children and women in developing and under developed countries. In developing world this nutritional disorder affects almost 3.6 billion people (ACC/SCN, 2000) and 30-60 % of women and children. Green leafy vegetables grow abundantly in India and are a precursor of vitamin A and a very good source of β-carotene (Premavalli et al., 2001). Leafy vegetables include fenugreek, spinach, coriander and mint etc which grows in abundance and have a short life span, in order to use this diet during off seasons preservation should be done properly to avoid wastage. There are so many techniques by which micronutrients in food can be enhanced. Fortification of food is one among such approaches by which the production of food and dietary diversification of micronutrients in foodstuff can be improved (Allen et al., 2006). According to USA Food and Drug (FDA) ferrous sulphate is regarded as a safe fortificant because of its bioavailability and is also used in bakery products as a fortificant. For baking and food processing industry ferrous sulphate is an excellent choice and can be mixed up with the flour to increase the bioavailability of micronutrients (Hurrell, 2000).

Spinach (*Spinacia oleracea* L.) is among the family of Amaranthaceae, an edible flowering plant (Le et al., 1999). Spinach are known to possess high levels of minerals that supports formation of nutrients for bones like calcium and magnesium; phosphorus; iron and a free radical-scavenger i.e it possess vitamin K; vitamin A; vitamin B; vitamin C and vitamin E. It also contains vitamin B6, vitamin B2, potassium, copper, protein, manganese, selenium, omega-3 fatty acids and naicin. It also contains antioxidants and adjusts blood pressure (Kuriyamaa et al., 2005).

About 90% of wheat is produced in India and is used for manufacturing of bakery products like bread, cake, muffins and biscuits (Bedekar 2001). Bread is a fermented bakery product and is mainly prepared by using a mixture of wheat flour, yeast, salt and water through a step wise procedure of mixing, kneading, proofing, shaping and baking (Dewettinck et al., 2008). World's half of the population consumes wheat flour as it is a staple food but still is an incomplete diet as it lacks micronutrients. For a large group of people in the world, the balanced diet is not easily available especially for those in developing countries. Green leafy vegetables are the only source of plant food that are cheap and are locally available. These green leafy vegetables are rich in micronutrients and can be incorporated during the development of breads that enhances the nutritive value of food. Therefore, attempts were made to prepare bread by incorporating wheat flour with ferrous sulphate powder (FeSO4) and dehydrated spinach powder to evaluate their effect on physico-chemical, microbiological, textural and sensory characteristics.

2. Materials and methods

2.1. Raw Materials

Fresh spinach (*Spinacia oleracea* L.) and food grade ferrous sulphate powder $(FeSO_4)^2.7H_2O$ were purchased from a local market in Srinagar, Kashmir. Samples were collected and stored at 4^{o}C till further use.

2.2. Preparation of the raw material

Spinach were cleaned manually, washed under tap water and oven dried at 55^{o}C. The dried spinach was ground into powder by using an electrical grinder (National brand, MX-895M model) and sifted through 125µm screen to obtain uniform size. The spinach powder (SP) was then stored in hermetically sealed packs at 4^{o}C for further use.

2.3. Bread Preparation

Bread was formulated according to Adeleke and Odedeji (2010) with slight modification. Wheat flour and FSP were used in the ratio of 0.025, 0.05, 0.10% and SP in the ratio of 1, 2.5 and 5% designated as T_0, T_1, T_2, T_3, T_4, T_5 and T_6 respectively. All ingredients were put together and mixed in a planetary mixer (Model SM-25, SINMAG Japan) for 2 min at 214 rpm and different substitution levels of ferrous sulphate powder and spinach powder were added and the content were mixed to obtain an elastic and smooth dough. The dough was then kept in a proofer (incubator) at a temperature of 37^{o}C for 1 hour. Afterwards dough was manually moulded and proofed for another 30 minutes and then knocked back to remove excess gas, rolled and moulded into pans. The pans loaded with breads were then placed in pre-heated baking oven at 225^{o}C for 30-35 min. After baking, the bread loafs were cooled for 1 hour at ambient temperature and then sliced and were packed in air tight polythene bags (LDPE) prior to analysis.

Table no: 1 Bread formulations

Whole wheat	150g
Yeast	3.0g
Sugar	6.0g
Salt	1.5g
Baking powder	2%
Ferrous sulphate	0.025, 0.05, 0.10%
Spinach powder	1,2.5,5 %

2.4. Compositional Analysis of SP and FSP

Moisture, fiber, ash, protein and crude fat content were determined by AOAC (2000) methods. Carbohydrate content of spinach powder and ferrous sulphate powder were determined by difference method that is by subtracting the sum of percentage of protein, crude fat, fiber, ash, and moisture content from 100.

2.5. Determination of Iron Content

The iron content in the samples was measured by using an atomic absorption spectrophotometer (AA700, Shimazdu) according to AOAC (1990) method. 1g sample was digested with 20 ml Di-acid (nitric acid and perchloric acid in the ratio of 3:1. The resulting digested sample was diluted with distilled water in a 50 ml calibrated flask. The solution was then used to determine iron.

Iron (mg/100g) = AAS reading (mg/L) × Dilution Factor (1)

2.6. Physical Evaluation of Bread

Loaf volume was calculated using rapeseed displacement method. Density and specific volume were determined by using the method of Feili et al., (2013) while as loaf height was measured using a meter rule.

Loaf volume (mL) = V_1-V_2 (2)

Where V_1 represent the volume of rapeseeds in the empty container (ml)

V_2 represents volume of the rapeseeds in the container containing sample (ml)

Specific volume was calculated as per the formula below

$$\text{Specific volume} = \frac{\text{loaf volume of bread (cm}^3\text{)}}{\text{weight of bread (g)}} \quad (3)$$

The density of bread was calculated as

$$\text{Density of bread} = \frac{\text{weight of bread (g)}}{\text{loaf volume of bread (cm}^3\text{)}} \quad (4)$$

2.7. Texture Profile Analysis (TPA).

Texture Analyser TA.HD.Plus (Stable Micro Systems, Godalming, Surrey, UK) was used to analyze the texture of bread crumb. The analysis was performed by using a cylinder probe (p/36) of 36mm radius. The force deformation curve was recorded when a compression cell of 5g and a compression distance of 25% was used. The speed of matrix was set at 1 mm s^{-1}, post-test speed of 10.0mm/s, test speed at 1.7mm/s. The test was determined after 24 hours of baking, 48 hours, 72 hours and 90 hours after storage at 22 ±1 °C and relative humidity of 45 ±1% (Xie et al. 2003).

2.8. Sensory Analysis

Sensory evaluation of bread samples fortified with different percentage of ferrous sulphate powder and spinach powder was carried by using 5 point hedonic scale. Consequently the bread samples were sliced (about 1.5cm thick), coded and served to semi-trained panel. Sensory panel recognized the attributes as sensory properties of bread like taste, color, texture and overall acceptability.

2.9. Microbial Evaluation

Total plate count (TPC), yeast and mold count in the bread samples were determined as per APHA (1976) method.

2.10. Statistical Analysis

One-way analysis of variance was used to analyze data in a randomized design with three replications and mean comparisons by using commercial statistical package SPSS (16.0, Chicago,IL).

Flow chart for the preparation of Bread

Raw materials
↓
Addition of water containing yeast
↓
Kneading (20 minutes)
↓
Proofing (1 hour at 37°C)
↓
Knock back
↓
Proofing (30 min)
↓
Baking (220 °C for 30-35 min)
↓
Cooling at ambient temperature (1 hour at 25±2°C)
↓
Slicing
↓
Packing in LDPE pouches
↓
Storage at ambient temperature (25±2°C)

3. Results and discussions

3.1. Compositional Analysis of Whole wheat flour and SP

The chemical analysis of wheat flour and spinach powder (SP) is depicted in Table 2. In order to get information about the overall proximate composition of any ingredients, analysis of chemical composition is important in determining the nutritional quality of the components being used in food. It doesn't only determine the chemical or nutritional status of food but also determines the shelf life and the production of designer foods for specific target groups. The shelf life of flour can be determined by its moisture content (9.50%) which is an important factor to define the quality parameter. At ambient temperature and at low moisture content of flour the storage stability is enhanced and thus increases its shelf life. Ash content of spinach powder (19.82%) measures the amount of mineral matter present in the powder. Protein, crude fiber, fat content of Whole Wheat flour and SP was found to be 11.20%, 2.50%, 1.43%, 19.82%, 4.92%, 3.32% respectively. Carbohydrate and Iron content in Whole Wheat flour and SP was also found to be 69.70%, 2.75 mg/100g iron, 12.01%, 11.75 mg/100g iron respectively (Riaz et al., 2007 and Chaturvedi et al., 2013).

Table 2. Compositional analysis of whole wheat flour (WWF) and Spinach Powder (SP)

Parameter	WWF	SP
Moisture (%)	9.50±0.02[a]	5.68±0.08 [b]
Protein (%)	11.20±0.04[b]	19.82±0.05[a]
Fat (%)	1.43±0.02[b]	3.32±0.14[a]
Ash (%)	1.67±0.01[b]	19.82±0.05[a]
Fiber (%)	2.50±0.01[b]	4.92±0.09[a]
Carbohydrate (%)	69.70±0.04 [a]	12.01±0.29 [b]
Iron (mg/100 g)	2.75±0.06[b]	11.75±0.21[a]

Values are mean of three replications ± standard deviation. Values followed by same small letter superscripts in a column do not differ significantly (P< 0.05)

3.2. Chemical Properties

Table 3 shows the effect of different substituents on the chemical composition of bread. Moisture and carbohydrate contents decreased with increased levels of SP. However, an increase in ash, protein, fibre and fat content was observed. This could be attributed due to the substitution effects caused by the fortificants. In case of bread fortified with FSP, moisture and fat contents decreased slightly with the increasing levels. Ash and protein content increased significantly (p≤0.05) (Akhtar et al. 2008).

The iron content significantly ($p \leq 0.05$) increased in both SP and FSP due to high levels of iron present in the fortificants.

Table 3. Effect of different levels of SP and FSP on the chemical composition of bread

Parameters	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Moisture (%)	35.20 ± 0.02^a	34.91 ± 0.01^a	$34.46^b \pm 0.12^b$	33.46 ± 0.04^b	35.18 ± 0.11^a	35.16 ± 0.02^a	35.04 ± 0.04^b
Protein (%)	9.07 ± 0.13^d	9.10 ± 0.03^c	9.33 ± 0.15^c	9.66 ± 0.11^c	9.11 ± 0.06^c	9.18 ± 0.04^d	9.24 ± 0.14^d
Fat (%)	1.51 ± 0.07^d	1.62 ± 0.04^c	1.73 ± 0.05^b	1.91 ± 0.01^a	1.49 ± 0.08^a	1.47 ± 0.06^a	1.44 ± 0.07^a
Ash (%)	1.10 ± 0.05^d	1.28 ± 0.06^c	1.51 ± 0.03^a	2.01 ± 0.07^a	1.95 ± 0.14^b	2.03 ± 0.06^a	2.08 ± 0.05^a
CHO (%)	$51.02^a \pm 0.03$	$50.52^b \pm 0.07$	$50.04^c \pm 0.06$	$49.00^d \pm 0.03$	$51.08^a \pm 0.08$	$51.15^a \pm 0.12$	$51.22^a \pm 0.02$
Iron (mg%)	2.72 ± 0.13^b	2.75 ± 0.03^a	2.79 ± 0.07^a	2.86 ± 0.05^a	2.73 ± 0.07^a	2.76 ± 0.05^a	2.78 ± 0.12^a

Values are mean of three replications ± standard deviation. Values followed by same small letter superscripts in a column do not differ significantly

T_0: whole wheat bread, T_1: Bread substituted with 1% SP, T_2: bread substituted with 2.5% SP, T_3: Bread substituted with 5% SP.T_4: Bread substituted with 0.025% FSP, T_5: bread substituted with 0.05%FSP, T_6: Bread substituted with 0.10% FSP.

3.3. Physical Characteristics

Physical parameters of fortified breads are listed in Table-4. The fortification of breads with SP and FSP significantly resulted in increased weight and density. The weight of FSP incorporated bread increased from 410.29 to 418.22g and the density increased from 0.63 to 0.65g/cm^3(Keetels et al., 1996). The effectiveness of gluten network decreases as the fortificant level increases, reducing the bread volume, height while increasing density (Feili et al., 2013).

Specific volume and loaf volume of breads fortified with SP and FSP decreased with increase in fortificant levels. SP and FSP cause dilution of gluten and subsequently affect the formation of optimal matrix of gluten during mixing, fermentation and baking process (Ragaee et al., 2011). Lower bread volume results due to partial replacement of wheat flour.

Table 4. Effects of spinach powder and ferrous sulphate powder incorporated on physical characteristics of bread

Physical characteristics	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Bread weight (g)	407.15 ± 0.07^a	420.51 ± 0.03^e	453.47 ± 0.13^f	480.54 ± 0.04^g	410.29 ± 0.03^b	413.83 ± 0.11^c	418.22 ± 0.02^d
Bread height (cm)	10.21 ± 0.04^f	9.64 ± 0.07^e	9.00 ± 0.06^b	8.91 ± 0.15^a	9.72 ± 0.08^e	9.56 ± 0.14^d	9.40 ± 0.11^c
Bread volume (cm^3)	650.25 ± 0.05^g	639.33 ± 0.03^c	630.01 ± 0.08^b	601.03 ± 0.05^a	645.37 ± 0.01^f	642.16 ± 0.06^e	640.25 ± 0.03^d
Density (g/cm^3)	0.62 ± 0.14^a	0.65 ± 0.02^a	0.71 ± 0.04^b	0.80 ± 0.08^c	0.63 ± 0.04^a	0.64 ± 0.02^a	0.65 ± 0.05^a
Specific volume (cm^3/g)	1.59 ± 0.04^c	1.52 ± 0.05^b	1.39 ± 0.08^b	1.25 ± 0.04^a	1.57 ± 0.09^c	1.55 ± 0.13^c	1.53 ± 0.02^c

Mean± SD with different superscripts in a column differ significantly ($P < 0.05$) (n= 3)

T_0: whole wheat bread, T_1: Bread substituted with 1% SP, T_2: bread substituted with 2.5% SP, T_3: Bread substituted with 5% SP.T_4: Bread substituted with 0.025% FSP, T_5: bread substituted with 0.05%FSP, T_6: Bread substituted with 0.10% FSP.

3.4 Sensory Evaluation

Table 5 evinced the result of hedonic test on different samples of breads. The bread enriched with SP was dark green in color than the control bread having slightly green color and has a negative approach on the acceptability of bread. This significant decrease in the colour of bread fortified with spinach powder ($T_1$to T_3) could be due to the chlorophyll present in the leaves that gives green color to bread (Abraham et al., 2013). Breads supplemented with FSP had slight blackish colour (Garby 1985) due to the precipitation caused by iron compounds when added to food and thus produces unacceptable changes in color and flavor of the product.

The taste, texture and overall acceptability of breads made by using different substitution levels of SP and FSP were non-significant with respect to the control. Higher levels of SP and FSP resulted in an unpleasant taste attributed by the herbal flavour of leaf powder (Abraham et al., 2013) and due to the slight metallic taste of iron (Saeed 2007). Higher levels of SP had negative influence on the texture. This decrease in texture might be due to slight coarser texture of spinach powder and reduction in the gluten contents of the bread (Sharma and Chauhan 2000). The results for sensory characteristics indicated that wheat flour bread incorporated with SP upto 1% (T_1) gives satisfactory overall consumer acceptability (Alam et al., 2013).

Table 5. Effect of spinach powder and ferrous sulphate powder on the sensory evaluation of breads

Attribute	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Colour	4.0	3.5	2.5	2.0	2.9	2.5	2.0
Taste	4.0	3.0	2.5	2.0	2.5	2.4	2.0
Texture	3.9	3.0	2.0	2.0	3.5	3.5	3.5
Overall acceptability	4	3.0	2.5	2.0	2.9	2.5	2.0

Mean± SDwith different superscripts in a column differ significantly ($P< 0.05$) (n= 3)
T_0: whole wheat bread, T_1: Bread substituted with 1% SP, T_2: bread substituted with 2.5% SP, T_3: Bread substituted with 5% SP.T_4: Bread substituted with 0.025% $FeSO_4$, T_5: bread substituted with 0.05%$FeSO_4$, T_6: Bread substituted with 0.10% $FeSO_4$.

3.5 Texture

The results for the texture analysis of the breads fortified with both SP and ferrous sulphate powder are presented in Table 6. Breads supplemented with spinach powder were significantly ($p\leq0.05$) harder than ferrous sulphate powder. This could be due to interaction between gluten and fibrous material (Gomez et al., 2003). Among treatment samples showed decreasing trend whereas increasing trend was followed throughout the period of storage. This increase in hardness could be due to effect of starch retrogradation (Paulina et al., 2012)

Results of statistical analysis of each sample for springiness and cohesiveness during storage and among treatments at same storage time can be found in Table 6. Springiness and cohesiveness significantly ($p\leq0.05$) decreased in all samples during storage. Intermolecular attractions are lost in between ingredients causing crumbling of crumb, and loss of water (Sharoba et al., 2013).Springiness was significantly higher in the spinach powder enriched samples as compared to ferrous sulphate incorporated bread. In composite breads the dilution of gluten structure minimizes the amount of gluten and thus reduces the elasticity in breads (Hoseney, 1994).

Gumminess and chewiness significantly increased during the entire storage period. However an increasing trend was observed among the treatments. Increase in hardness during storage results in retrogradation properties of wheat flour as reported previously by Sharoba et al., (2013) Wang et al., (2002).

Table 6. Texture profile analysis (TPA) of iron fortified bread during storage

Treatments	Storage (days)				Treatment Mean
	0	2	4	6	
Hardness					
T_0	8.20^{aD}	8.75^{bC}	9.20^{cC}	9.42^{cD}	**8.89^{C}**b
T_1	7.83^{aC}	8.20^{bC}	9.01^{dC}	9.36^{eD}	**8.60^{Cc}**
T_2	6.00^{a}	6.41^{bA}	6.98^{cA}	7.20^{cB}	**6.63^{Ab}**
T_3	5.94^{Aa}	6.56^{bA}	7.62^{dB}	8.01^{eC}	**7.03^{Bc}**
T_4	6.20^{Aa}	6.57^{bA}	6.81^{bA}	6.84^{bA}	**6.60^{Ab}**
T_5	6.15^{Aa}	6.52^{bA}	6.^{73Ab}	6.82^{bA}	**6.58^{Ab}**
T_6	6.09^{Aa}	6.45^{bA}	6.^{68Abc}	6.78^{Aa}	**6.51^{Ab}**
Storage Mean	**6.63^{Ba}**	**7.07^{Bb}**	**7.57^{Bc}**	**7.77^{Cc}**	
Springiness					
T_0	0.94^{Ba}	0.92^{Ba}	0.90^{Ca}	0.88^{Ba}	**0.90^{Aa}**
T_1	0.91^{Ba}	0.87^{Ba}	0.85^{BCa}	0.84^{Ca}	**0.86^{Aa}**
T_2	0.90^{Bb}	0.88^{Bab}	0.86^{Ba}	0.81^{ABC}	**0.85^{Aa}**
T_3	0.86^{Ac}	0.81^{Abc}	0.77^{Ab}	0.68^{Aa}	**0.79^{Ab}**
T_4	0.92^{Bb}	0.91^{Bb}	0.85^{Ba}	0.80^{ABa}	**0.86^{Aab}**
T_5	0.87^{ABb}	0.85^{Ab}	0.79^{Aa}	0.73^{ABa}	**0.81^{Aab}**
T_6	0.80^{Ab}	0.77^{Aab}	0.71^{Aa}	0.69^{Aa}	**0.74^{Aa}**
Storage Mean	**0.88^{Bb}**	**0.85^{Ba}**	**0.81^{Ba}**	**0.76^{Aa}**	
Cohesiveness					
T_0	0.72^{Bb}	0.70^{Cb}	0.62^{Ca}	0.59^{Ca}	**0.66^{Bab}**
T_1	0.67^{ABb}	0.63^{BCb}	0.55^{BCa}	0.51^{BCa}	**0.59^{Bab}**
T_2	0.65^{Ac}	0.54^{ABb}	0.48^{Bab}	0.43^{ABa}	**0.52^{Ab}**
T_3	0.61^{Ac}	0.47^{Ab}	0.39^{Aa}	0.38^{Aa}	**0.46^{Aab}**
T_4	0.69^{Bc}	0.54^{ABb}	0.45^{Aa}	0.48^{Ba}	**0.51^{Aab}**
T_5	0.62^{Ac}	0.60^{Bb}	0.58^{Cb}	0.39^{Aa}	**0.52^{Ab}**
T_6	0.59^{Ac}	0.52^{Abc}	0.47^{ABb}	0.36^{Aa}	**0.48^{Ab}**
Storage Mean	**0.65^{Ac}**	**0.56^{Bb}**	**0.49^{Ba}**	**0.43^{ABa}**	
Chewiness					
T_0	3.62^{DEa}	4.25^{Eb}	5.05^{Ed}	5.92^{Fe}	**4.71^{Dc}**
T_1	2.89^{BCa}	3.32^{Bb}	4.45^{Dd}	4.08^{Bcd}	**3.97^{Cc}**
T_2	2.03^{Aa}	3.27^{Bb}	3.92^{BCc}	4.20^{Bd}	**3.35^{Bb}**
T_3	1.98^{Aa}	2.61^{Ab}	3.03^{Acd}	3.36^{Ad}	**2.67^{Abc}**
T_4	3.61^{Da}	3.82^{Cab}	4.45^{Dcd}	5.14^{Ed}	**4.06^{Dbc}**
T_5	3.04^{Ca}	3.98^{Cb}	4.39^{Dc}	4.84^{Dd}	**4.25^{Dbc}**
T_6	3.72^{Ea}	3.91^{Ca}	4.27^{CDb}	4.39^{BCb}	**4.07^{Dab}**
Storage	**3.04^{Ca}**	**3.59^{BCb}**	**4.18 ^{Cc}**	**4.66^{CDc}**	

Mean					
Gumminess					
T_0	3.15^{Da}	3.62^{Dbc}	4.66^{Fc}	5.58^{Ed}	**4.25^{Dc}**
T_1	2.52^{Ca}	3.63^{Db}	3.95^{DEc}	4.36^{Cd}	**3.61^{BCb}**
T_2	1.92^{Aa}	2.18^{Aa}	2.86^{Ac}	3.08^{Ac}	**2.51^{Ab}**
T_3	2.04^{Aa}	2.92^{Bc}	3.21^{Bc}	3.62^{Bd}	**2.39^{Ab}**
T_4	2.82^{Ca}	3.52^{Db}	4.15^{Ec}	4.87^{Dd}	**3.76^{Cb}**
T_5	2.64^{Ca}	2.82^{Ba}	3.69^{CDc}	4.53^{Cd}	**3.67^{Bbc}**
T_6	2.45^{Ca}	3.19^{Cb}	3.62^{Cc}	4.32^{Cd}	**3.39^{Bbc}**
Storage Mean	**2.50^{Ca}**	**3.12^{Cb}**	**3.73^{Dc}**	**4.33^{Cd}**	

Means with different superscripts in a column differ significantly ($P< 0.05$) ($n= 3$)

T_0: whole wheat bread, T_1: Bread substituted with 1% SP, T_2: bread substituted with 2.5% SP, T_3: Bread substituted with 5% SP. T_4: Bread substituted with 0.025% $FeSO_4$, T_5: bread substituted with 0.05% $FeSO_4$, T_6: Bread substituted with 0.10% $FeSO_4$.

4.6 Microbiological studies

Total plate count, yeast and mould count of breads fortified with SP and FSP were performed at 0, 2, 4 and 6th day of storage and are presented in Table-7. There was an increase in total plate count (0.14 to1.45× 10^2cfu/g) and yeast and mould count (1.4 × 10^1 to 2.7×10^1cfu/g) with increase in storage. However, with increase in the levels of fortification there was a decrease in total plate count and Yeast and mold count. Increasing the levels of fortification, there was a decrease in water activity which in turn decreases the microbial load. During storage an increase in the water activity of bread favors the growth of bacteria. However, microbial load remained within the permissible limits (Smith et al., 2004).

Breads fortified with FSP showed lower microbial count than that of spinach incorporated breads. This could be due to the high mineral content which can possibly inhibit the growth of microbes. As the minerals are hygroscopic in nature, they can stop the microbial growth by decreasing the available moisture (Akhtar et al. 2008).

Table 7. Effect of ambient storage (21-25 °C) on microbiological characters of wheat bread supplemented with various levels of spinach powder and ferrous sulphate

Treatments	**Storage (days)**					
		0	**2**	**4**	**6**	**Treatment mean**
Total plate count (cfu/g)	T_0	TLTC	$0.22^{Aa}\times10^2$	$0.30^{Aa}\times10^2$	$1.35^{Ac}\times10^2$	$0.46^{Ab}\times10^2$
	T_1	TLTC	$0.19^{Aa}\times10^2$	$0.26^{Aa}\times10^2$	$1.33^{Ac}\times10^2$	$0.44^{Ab}\times10^2$
	T_2	TLTC	$0.16^{Aa}\times10^2$	$0.22^{Aa}\times10^2$	$1.32^{Ab}\times10^2$	$0.42^{Aa}\times10^2$
	T_3	TLTC	$0.13^{Aa}\times10^2$	$0.20^{Aa}\times10^3$	$1.29^{Ab}\times10^2$	$0.40^{Aa}\times10^2$
	T_4	TLTC	$0.14^{Aa}\times10^2$	$0.18^{Aa}\times10^2$	$1.20^{c}\times10^2$	$0.38^{Ab}\times10^2$
	T_5	TLTC	$0.11^{Aa}\times10^2$	$0.15^{Aa}\times10^2$	$1.17^{Ac}\times10^2$	$0.35^{Ab}\times10^2$
	T_6	TLTC	$0.10^{Aa}\times10^2$	$0.12^{Aa}\times10^2$	$1.14^{Ac}\times10^2$	$0.34^{Ab}\times10^2$
Storage Mean	**TLTC**		**$0.14^{Aa}\times10^2$**	**$0.20^{Aa}\times10^2$**	**$1.45^{Ab}\times10^2$**	
Yeast and mold count (cfu/g	T_0	TLTC	$2.2^{Ba}\times10^1$	$2.7^{Db}\times10^1$	$3.2^{Cc}\times10^1$	$2.05^{Ba}\times10^1$
	T_1	TLTC	$2.0^{Ba}\times10^1$	$2.4^{CDb}\times10^1$	$3.0^{Bc}\times10^1$	$1.85^{Ba}\times10^1$

	T_2	TLTC	$1.19^{Aa}\times10^{1}$	$2.3^{Cc}\times10^{1}$	$2.8^{Bd}\times10^{1}$	$1.57^{Ab}\times10^{1}$
	T_3	TLTC	$1.16^{Aa}\times10^{1}$	$2.0^{BCc}\times101$	$2.8^{Bd}\times10^{1}$	$1.49^{Ab}\times10^{1}$
	T_4	TLTC	$1.14^{Aa}\times10^{1}$	$1.9^{Ac}\times10^{1}$	$2.9^{Bd}\times10^{1}$	$1.55^{Ab}\times10^{1}$
	T_5	TLTC	$1.11^{Aa}\times10^{1}$	$1.7^{Ab}\times10^{1}$	$2.6^{ABc}\times10^{1}$	$1.35^{Aa}\times10^{1}$
	T_6	TLTC	$1.10^{Aa}\times10^{1}$	$1.7^{Ac}\times10^{1}$	$2.2^{Ad}\times10^{1}$	$1.25^{Ab}\times10^{1}$
Storage Mean		**TLTC**	$\mathbf{1.4^{Aa}\times10^{1}}$	$\mathbf{2.1^{Cb}\times10^{1}}$	$\mathbf{2.7^{Bc}\times10^{1}}$	

Means with different superscripts in a column differ significantly ($P < 0.05$) ($n = 3$)

T_0: whole wheat bread, T_1: Bread substituted with 1% SP, T_2: bread substituted with 2.5% SP, T_3: Bread substituted with 5% SP.T_4: Bread substituted with 0.025% $FeSO_4$, T_5: bread substituted with 0.05%$FeSO_4$, T_6: Bread substituted with 0.10% $FeSO_4$.

4. Conclusions

This study was carried out to investigate the effect of fortification of spinach powder on physicochemical, textural and microbiological properties of bread. Six different treatments of the product with substitution of *Spinacea oleracea* powder, and $FeSO_4$ at levels of 1, 2.5, 5 and 0.025, 0.05, 0.10 percent respectively were used in the development of iron fortified breads. The study also revealed that ash content increased significantly with the increasing level of ferrous sulphate while as moisture, protein, fat and fiber decreased with increase in supplementation of ferrous sulphate. The studies revealed that the ash, protein, fiber and fat increased significantly with the increasing level of *Spinacea oleracea* powder, while as moisture and carbohydrate decreased significantly with the increase in spinach powder. The physical characteristics showed significant increase in weight and density of bread while as decreasing trend in bread volume, bread height and specific volume was observed. The scores for overall acceptability of the fortified bread samples decreased by increasing the levels of fortificants. The result obtained revealed that the Bread prepared by using 1% level of Spinach powder showed better composition in terms of physicochemical properties. Prepared products were stored at ambient temperature (25^{o}C) in LDPE pouches. The storage depicted significant increase in textural characteristics while a decreasing trend in the mean values between the treatments was observed. Hardness, chewiness and gumminess of bread samples increased during the storage from 6.63-7.77 N, 2.49-4.14 mm and 3.04-4.66 respectively while the springiness and cohesiveness of bread samples decreased from 0.88-0.76 mm and 0.65-0.43 mm respectively. Total plate count and yeast and mould count showed slightly decreasing trend within treatments while an increasing trend was observed during the storage from 0-1.14×10^{2} cfu/g and 0 to 2.2×10^{1} cfu/grespectively. This decreasing trend during the storage was found within the permissible limits.

Deficiency of iron is relatively the most common nutritional problem throughout world that affects mainly all the age groups especially women, infants between 9 and 12 months and children under 5 years. In developing countries iron deficiency can affect 30-40% of growing children and women having premenopausal and it can be corrected successfully by population basis. Deficiency of iron can be prevented by increasing the bioavailability and taking iron rich diet.

Current study suggests the possibility of using spinach powder and ferrous sulphate powder as an interventional study to control iron deficiency anemia. The nutritional content of the breads increased but got poor appearance with increasing levels of fortificants. The breads can be improved by using some bleaching and improving agents to increase their preference/acceptability.

5.References

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