International Journal of Chemical Studies 2020; 8(3): 579-584

International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(3): 579-584

Received: 16-03-2020
Accepted: 18-04-2020

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Physico-chemical and sensory properties of HTST Pasteurized milk fortified with casein hydrolysate-iron complex

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DOI: https://doi.org/ 10.22271/chemi.2020.v8.i3g.9272

Abstract
This study investigated the effect of Buffalo Casein Hydrolysate-Iron complex (BCH-Iron) on fortification in milk. The high temperature sort time (HTST) pasteurized milk was fortified with 30 ppm iron using BCH-Iron complex (1.4 mg Fe/ml). The effect on physicochemical and sensory properties of HTST pasteurized milk was evaluated for 7 days. During storage non-significant (p>0.05) difference in sensory properties of BCH-Iron complex fortified milk observed compare to control. TBA value were significantly (P<0.05) higher in $FeSO_4$ fortified milk and the Colour profile of BCH-Iron complex fortified milk was significantly (P<0.05) different then control and $FeSO_4$ fortified HTST pasteurized milk.

Keywords: Casein hydrolysate-iron complex, HTST, pasteurized milk, TBA, sensory, heat stability

Introduction

Iron deficiency is usually the result of insufficient dietary intake of iron, poor utilization of iron from the ingested food or a combination of the two (Gaucheron, 2000) [9]. Iron absorption depends not only on the amount consumed but also its bioavailability, which in turn is affected by the different components of dietary foods. Dietary iron is usually poorly absorbed especially from plant-based foods because of the high content of phytate, which is an important inhibitor of iron absorption (Fredlund *et al*., 2006) [7]. Thus, iron deficiency is common in populations consuming plant-based diet.

To combat iron deficiency, fortification of food with iron is a valid and well known technology as a part of food-based approach when and where existing food supplies and limited access fail to provide adequate levels of iron in the diet (Hurrell, 2002) [11]. Beside that fortification of food is a major strategy by which the nutrient quality and quantity of the food can be enriched (Gahruie *et al*., 2015) [8]. But food fortification by using the suitable fortificant without affecting the quality characteristics of finished product and ensuring higher bioavailability remain a challenging task. Fortification of iron in food is associated with number of difficulties including change of colour of food by forming complexes with compounds such as sulphur compounds, tannins, and polyphenols. Iron is chemically reactive under alkaline conditions, gives a metallic taste, cause gastrointestinal discomfort and gives an unpleasant flavor due to fat oxidation (Chen and Oldewage-Theron, 2002; Mehansho, 2006) [4, 16].

Milk and milk products like yogurt are most nutritious foods and possesses great biological value and digestibility. And are rich in calcium, vitamin D, phosphate, conjugated linoleic acids and proteins. However, milk has an extremely low content of iron, thus, the fortification of iron into milk and milk products, can make it more nutritious for anemic people (El-Kholy *et al.*, 2011) [6] and considered as practical and cost-effective long term solution (Abbasi and Azari, 2011) [1]. Recent trend of increased demand for ultra-high temperature processed milk throughout the world (Abdulghani *et al*., 2015) [2] and increased consumption of fermented health based products makes UHT milk and yogurt ideal product for iron fortification (Santillan-Urquiza *et al*., 2017) [21]. The dairy products quality either sensory, physico-chemical, rheology or microbial can greatly depends on the type, source and quantity of fortificant used thus it is utmost to find the alternative to minimize the adverse effect on the product and simultaneously maximizing the absorption and without sacrificing the quality.

The micronutrient like iron zinc, magnesium, phosphorus can be present in milk in the form of free ion or complexes with various milk component like carbohydrates, protein, lactose, amino acids etc. Further between caseinate and whey protein isolate, the affinity of caseinate to bind iron was found higher than that of whey proteins (Sugiarto *et al*., 2010) [22].

Iron- peptide complexes and amino acid chelates reported to possess greater solubility, digestibility and absorption rate, thus are required at a lower supplementation rate with an equivalent or improved effects compared to inorganic salts alone (Peres *et al*., 1999) [17]. The peptide involved in complexation, possesses appreciable antioxidant power, reported to relieve stress during anemia. Chelated iron also prevents iron induced oxidation of different food moieties via Fenton reaction (Taylor and Richardson, 1980; Sugiarto *et al*., 2010) [23, 22]. Limited information is available on iron fortification in milk through the use of casein hydrolysate iron complex. Very few reports are available on changes in physico-chemical properties of milk and milk products fortified with hydrolysate-iron complex.

Materials and methods

Milk sample and reagent

Milk was collected from the experimental dairy of National Dairy Research Institute, Karnal, Haryana, India, and standardized to 3% fat and 8.5% solid-not-fat (SNF). $FeSO_4.7H_2O$ (Sigma Chemical Co., St. Louis, MO, USA) was used for this study. All the reagents used for the study were of "AR" grade.

Preparation of BCH-Iron complex

Buffalo sodium caseinate (5 % w/v) was hydrolyzed by Flavourzyme at enzyme to substrate ratio i.e. 214.70 units/g casein under hydrolysis conditions as 7.5 pH, 55°C temperature and 3 hr. hydrolysis time. Followed by enzyme inactivation in boiling water for 10 min and centrifugation in refrigerated centrifuge (Kubota, Tokyo) at 10000 rpm and 4°C (to remove insoluble content). The supernatant collected and stored at -20 °C for further use, followed by 0.22 µ filtration of the hydrolysate and then the permeate hydrolysate solution was subjected to complexation treatment with iron under pH 8, temperature 60˚C and time for 3 hr. After completion of reaction was subjected to centrifugation at 800xg for 20 min to remove insoluble part. The soluble part was further stored at -20˚C and Total iron was determined by Atomic absorption spectrometer (AA-700, Shimadzu, Japan) and free iron (iron chelation) was estimated by ferrozine method (Gu *et al*., 2010) [10]. The hydrolysis of the buffalo caseinate resulted in to degree of hydrolysis (DH) corresponding to 28.3% and iron chelation using flavourzyme as 600.25 µg $FeSO_4$ .7H2O binding/ mg peptide.

The BCH-Iron complex prepared by using 40 mg/ml hydrolysate concentration, which corresponded to 1.4 mg/ml iron content, was selected for fortification of dairy products

Iron source addition

Calculated amount of iron source (BCH-Iron complex and $FeSO_4.7H_2O$) were added to obtain 30 ppm level of iron in HTST pasteurized milk. Proper mixing was done to obtain uniform distribution of salts.

Preparation of iron fortified milk

Homogenized toned milk was taken in clean and dried stainless steel vessel and the calculated amount of BCH-Iron complex and iron salt were added. Addition was accompanied by thorough mixing for 10 minutes with the help of stirrer for the complete dissolution of fortificant, followed by HTST pasteurization (83 °C/16 sec) and immediately cooling and storage at 4 °C under refrigeration condition to evaluate sensory and physiological changes during storage of 7 days.

Sensory evaluation of HTST pasteurized milk

Sensory evaluation was done by a panel of seven trained judges. Composite scoring card for sensory analysis of HTST pasteurized milk as suggested by BIS (IS: 7768 1975) were used, with slight modifications. Maximum score of 10 for colour and appearance, 20 for odour, 40 for taste and 30 for mouth feel, thus a total of 100 points. In flavour characteristics, the main focus was on metallic, rancid and oxidized flavour. Other mentioned flavour characteristics were excluded. Saline water (0.89% sodium chloride solution, at room temperature) was provided as palate cleanser for rinsing mouth and cleaning the tongue before testing each sample.

Colour profile of HTST pasteurized milk

Hunter colourimeter was used to measure the colour of fortified, thermally processed milk samples and to monitor degree of change in colour during storage produced by addition of iron salts to milk. The colour coordinates of this meter were L=whiteness; a=redness to greenness and b=yellowness to blueness. The instrument was calibrated with standard reference tile, coordinates for the tile were L*=50.83 to 93.00; a*= 0.92 to -26.27 and b*= 1.70 to 12.12. Milk samples were brought to room temperature and colour measurements were performed.

Physico- chemical analysis of HTST pasteurized milk

pH, acidity and viscosity

pH of control and fortified milk samples, was determined by pH meter (PHAN, Lab India, India) as per the method described in IS: SP:18, part XI (1981). The pH meter was first calibrated using standard buffers of pH 4.0 and 9.2 and standardized using pH buffer of 7.0 at 20.0±0.10C. Titratable acidity of control and fortified milk was determined as per IS: SP: 18, Part XI (1981). The viscosity of the milk samples was determined by capillary flow ostwald viscometer.

Heat stability

The heat stability of control and fortified samples were determined as heat coagulation time (HCT) according to the method of Davies and White (1966) [5] as modified by Jairam *et al*. (1976) [14].

For the determination of heat stability of individual milk samples, 2ml of milk from each lot was taken in corning glass tube (10 cm in length with 8 mm internal diameter) in duplicates and corked at both ends with silicone rubber corks. The tubes were then rocked at the rate of 8 cycles per minute in a metal carriage inside the hot paraffin oil (140±10C) in a thermostatically controlled bath. The heat coagulation time (HCT) in minutes was recorded as the time elapsed between the moment the tubes were dipped into oil and appearance of first visible clot.

Estimation of TBA value of fortified milk

TBA value of milk was determined as per the method described by King (1962). Milk samples were warmed to 300C and accurately 17.6 ml of milk pipetted into a flask fitted with glass stoppers. 1 ml of TCA solution (containing 1g TCA/ml) was added followed by 2 ml of 95% ethanol. The flask was stoppered and contents were shaken vigorously for 10 seconds. After 5 minutes, contents were filtered through Whatman filter paper no. 42. 4 ml of clear filtrate was taken in a test tube, 1ml of TBA reagent (made by dissolving 1.4 g of 2-TBA in 100 ml 95% ethanol) was added. The contents were stoppered, mixed and placed at 600C in a water bath for 60 minutes. After cooling to room temperature, optical density was spectrophotometrically recorded at 532 nm. Reagent blank was also tested simultaneously replacing distilled water with milk. TBA values of samples were expressed as optical density (OD) at 532 nm.

Estimation of iron content of fortified milk

Iron was analyzed using atomic absorption spectrophotometer (AAS) by AOAC (2005) [3] using dry digestion method.

Microbiological analysis

The thermally processed milk samples were examined for microbiological parameters according to the methods described in IS: SP 18 (Part I, 1980).

Results and discussion

Effect of BCH-Iron complex fortification on sensory parameter of HTST pasteurized milk during storage at 4 °C

Iron salts had been reported to undesirably affect the sensory properties of fortified foods. The addition of the iron fortificant is expected to affect the organoleptic quality of fortified milk; therefore milk samples were evaluated for sensory parameters namely flavour, colour & appearance, odour and mouth feel using a structure composite score card by semi- trained panelists.

Data are presented as mean±SEM (n=3), ^{ab,} Means with different superscripts within columns differ significantly ($P<0.05$). ^{AB,} Means with different superscripts within rows differ significantly ($P<0.05$)

Effect on Colour and Appearance

The colour and appearance attribute of BCH-Iron complex fortified HTST pasteurized milk showed reddish colour & appearance unlike $FeSO_4$ fortified HTST pasteurized milk, which retained whiteness similar to control milk sample. The score value based on colour attribute significantly ($P<0.05$) decreased on 7th day.

Effect on Taste

During the storage period of 7 days taste scores of control as well as fortified milk lowered significantly from initial average score at the 5th day of storage. The milk fortified with BCH-Iron complex and ferrous sulfate did not differ significantly (p>0.05) in their taste scores compared to control milk sample but relatively lower score was observed during storage as compared to control.

Effect on Odour

Odour score of iron salt fortified milk had non-significant ($P<0.05$) difference compare to the control milk sample during the storage. The odour score of control, BCH-Iron complex and $FeSO_4$ fortified HTST pasteurized milk from 1st day to 7th day varied from 18.58±0.20 to 16.58±0.20, 17.83±0.40 to 15.83±0.27 and 17.50±0.34 to 15.83±0.21, respectively. The results were in agreement with the Sachdeva *et al*., (2015) who studied the impact on physico-chemical properties of iron and vitamin A fortification of pasteurized toned milk and reported that the decrease in odour score of the treated samples might be due to formation of metallic and/or oxidized taint during storage of the HTST pasteurized milk.

Effect on Mouth feel

Mouth feel scores too followed the similar trend, but the lowering in mouth feel score was higher and significant after 5th day on addition of BCH-Iron complex and $FeSO_4$ fortificant. The mouth feel score of control, BCH-Iron complex and $FeSO_4$ fortified HTST pasteurized milk on 1st day to 7th day were 27.33±0.49 to 24.91±0.32, 25.66±0.33 to 23.75±0.21 and 26.66±0.49to 24.16±0.21, respectively.

Effect on total score

Total score of HTST pasteurized milk fortified with BCH-Iron complex was significantly ($P<0.05$) different from control milk throughout the storage but the major attributes affected were colour and taste which might be due to the reddish colour and the bitterness of hydrolysate of the BCH-Iron complex used for fortification of the milk. From the data presented in Table 1, the sensory score of BCH-Iron complex and $FeSO_4$ fortified HTST pasteurized milk was significantly ($P<0.05$) different from control milk but the reduction of score in the BCH-Iron complex HTST pasteurized milk was due to the colour attributes and thus by masking the colour and taste attributes, the sensory score can be improved.

Colour profile of HTST pasteurized milk

Colour attributes of control and fortified milk were determined as L* (Whiteness), a* (Redness) and b* (yellowness) using COLOURFLEX hunter colourimeter.

Effect on L* value

The result showed that L* value decreased in all the samples during the storage period and the lowering in whiteness value was significantly ($P<0.05$) different. However addition of ferrous sulphate as fortificant did not cause any significant difference in L* value, when compared to control samples, whereas addition of BCH-Iron complex lowered the L* value significantly ($P<0.05$).The difference between control (86.71±0.050 on 1st day) and ferrous sulfate samples (86.42±0.029 on 1st day) were not significant compared to BCH-Iron complex (82.37±0.024 on 1st day). The increase in L* value observed throughout the storage. Lowering in L* value of BCH-Iron complex fortified HTST pasteurized milk might be because of colour imparted by BCH-Iron complex.

Effect on a* value

a* value(Redness) of control and ferrous sulfate fortified milk were giving negative value while the BCH-Iron complex fortified sample showed positive value from the initial day to final day of storage. While the a* value in all treated sample during storage was significant ($P<0.05$).

The difference in between control HTST pasteurized milk (-1.51±0.030 on 1st day to -1.28±0.020 on 7th day) and ferrous sulfate samples fortified HTST pasteurized milk (-1.67±0.015 on 1st day to -1.39±0.049 on 7th day) were not significant compared to BCH-Iron complex fortified HTST pasteurized milk (1.21±0.015 on 1st day to 1.03±0.027 on 7th day), this could be because of red colour imparted by BCH-Iron complex, which was in brown Colour form thus giving less whiteness compare to control and $FeSO_4$ fortified HTST pasteurized milk.

Effect on b* value

As shown in the Table 2, the b* value (Yellowness) of BCH-Iron complex fortified milk sample (13.35±0.021 on 1st day) showed significant ($P<0.05$) difference compare to control (10.22±0.049 on 1st day) and $FeSO_4$ fortified HTST pasteurized milk (10.24±0.013 on 1st day). This indicates yellow tinge imparted by BCH-Iron complex. The b* value of all treated sample throughout the storage remain unaffected by level of fortification.

Effect of Iron Fortification on physicochemical properties of HTST pasteurized milk during storage at 4 ^{0}C

Effect on acidity

Results as presented in Table 3 Shows that acidity of fortified HTST pasteurized milk increased during the storage under refrigerated conditions. Similar trend was noted in case of control sample. Significant ($P<0.05$) increase in acidity was observed on or after 3rd day of storage in control as well as fortified milk samples. Thus the results indicated that fortification of iron did not influence the acidity of HTST pasteurized milk and no significant ($p>0.05$) difference was observed among the control and fortified milk samples.

Effect on pH

pH of $FeSO_4$ fortified samples decreased significantly ($P<0.05$) upto 5th day compared to control and bch-iron sample. This might be due to release of micellar bound H^+ ions. Saini *et al.* (1987) [20] and Rosenthal *et al.* (1993) [18] reported that there was decrease in pH of milk samples containing iron. While initial pH of bch-iron had similar trend as control sample, however, a significant decrease in pH was observed in $FeSO_4$ fortified samples. A progressive decrease in pH of control as well as fortified samples was observed during storage period.

Data are presented as mean±SEM (n=3), ^{ab,} Means with different superscripts within columns differ significantly ($P<0.05$). ^{AB,} Means with different superscripts within rows differ significantly ($P<0.05$)

Effect on viscosity

Viscosity of milk samples was not influenced by the fortification of BCH-Iron complex or ferrous sulfate salt. Trend of viscosity in milk samples was not linear during storage.

Effect of iron fortification on heat stability of milk

The heat stability was measured as HCT of milk (BCH-Iron complex, FeSO4 fortified and control) after adjusting the pH between 6.4 to 7.0 at intervals of 0.1 unit using disodium hydrogen phosphate and sodium dihydrogen phosphate along with processing at its natural pH was studied and the results are presented in fig. 1.

HCT/pH profile of control milk sample at its natural pH (6.6) was observed to maximum corresponding to 31.40±0.20 minutes at 140±10C while that of milk fortified with BCH-Iron complex milk and FeSO4 milk at 30 ppm was found to be 28.75±0.75 and 28.70±0.2minutes, respectively. There was a significant difference ($P<0.05$) in HCT of iron fortified milk as compared to control, although no significant difference ($p>0.05$) between BCH-iron fortified and FeSO4 fortified milk was observed. A minima of HCT/pH curve in the pH region 6.8 -7.0 was observed. The data revealed that addition of iron salt of BCH-Iron complex caused significant ($P<0.05$) reduction in heat coagulation time as compared to control, although no significant difference was observed compared to $FeSO_4$ fortified milk.

Effect on Thiobarbituric acid (TBA)

Unlike hydrolytic rancidity expressed FFA, oxidative rancidity of milk fat determined as Thiobarbituric acid value and expressed as Optical Density (OD) at 532 nm, enhanced during storage of HTST pasteurized control and fortified milk. Increase in oxidative rancidity was significantly affected by storage period and iron content. Incorporation of bch-iron and $FeSO_4$ in milk resulted in rise in TBA value during storage; however, rate of increase in TBA value was highest in $FeSO_4$ fortified milk sample compare to bch-iron and control. The TBA value obtained during storage shows significant difference throughout storage period and also in between the fortificant.

Estimation of iron content of fortified milk

The level of iron content in fortified milk using BCH-Iron complex and $FeSO_4$ was validated corresponding to 30 ppm using atomic absorption spectrometer (Table 4).

Effect of fortification on microbiological parameter of HTST pasteurized milk during storage at 4-7 °C

Standard plate counts stated as log cfu per ml were in the range of 3.85 that increased to 5.28 on the 7th day of storage (Table 5). The increase after 3rd day in SPC was observed and it is apparent that increasing the iron level as fortificant also caused slight increase in SPC. Iron is one of the essential elements required for various cellular functions of microbes and also protects them from the adverse conditions. Higher availability of iron in milk could have resulted in lower destruction of bacteria. However, coliform and yeast & mold counts were remained nil throughout the storage. Absence of coliforms particularly indicates that desired hygiene measures were applied during the entire production process and also pre and post production handling of milk.

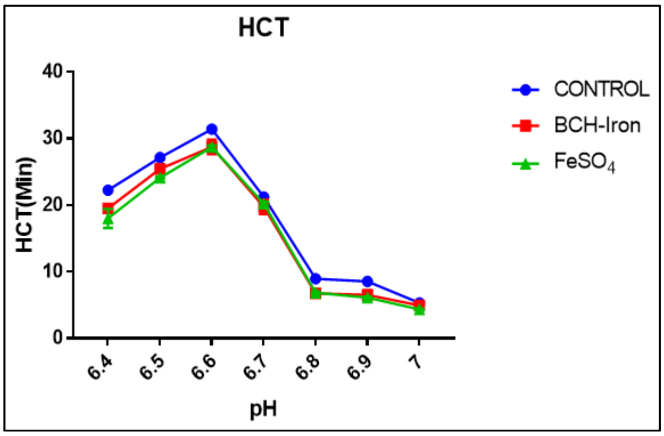

Fig 1: HCT/pH graph of control, BCH-Iron complex and $FeSO_4.7H_2O$ fortified milk

Table 1: Effect of BCH-Iron complex on the sensory score of HTST pasteurized milk during storage at 4 °C

Days		1	3	5	7
Color & appearance	Control	8.58±0.20^{Aa}	8.33±0.16^{Aa}	7.75±0.17^{Ba}	7.16±0.21^{Ba}
	BCH-Iron	8.16±0.30^{Aa}	7.91±0.27^{Aa}	7.16±0.24^{Ba}	6.33±0.16^{Bb}
	$FeSO_4$	8.50±0.22^{Aa}	8.00±0.25^{Aa}	7.25±0.25^{Ba}	6.75±0.28^{Ba}
Odour	Control	18.58±0.20^{Aa}	18.00±0.36^{Aa}	17.58±0.37^{Aa}	16.58±0.20^{Ba}
	BCH-Iron	17.83±0.40^{Aa}	17.26±0.41^{Aa}	16.83±0.49^{Aa}	15.83±0.27^{Ba}
	$FeSO_4$	17.50±0.34^{Aa}	17.16±0.30^{Aa}	16.66±0.30^{Aa}	15.83±0.21^{Ba}
Taste	Control	36.66±0.49^{Aa}	35.33±0.33^{Aa}	35.16±0.49^{Aa}	33.50±0.50^{Ba}
	BCH-Iron	35.08±0.58^{Aa}	34.25±0.42^{Aa}	34.08±0.45^{Aa}	32.58±0.53^{Ba}
	$FeSO_4$	35.16±0.65^{Aa}	34.00±0.28^{Aa}	34.16±0.38^{Aa}	32.66±0.51^{Ba}
Mouthfeel	Control	27.33±0.49^{Aa}	26.66±0.33^{Aa}	25.91±0.37^{Ba}	24.91±0.32^{Ba}
	BCH-Iron	25.66±0.33^{Ab}	25.66±0.33^{Aa}	25.00±0.36^{Aa}	23.75±0.21^{Bb}
	$FeSO_4$	26.66±0.49^{Aa}	26.58±0.27^{Aa}	25.16±0.30^{Ba}	24.16±0.21^{Ba}
Total	Control	91.16±0.71^{Aa}	88.33±0.85^{Ba}	86.41±0.72^{Ca}	82.16±0.70^{Da}
	BCH-Iron	86.75±0.57^{Ab}	85.10±0.60^{Ab}	83.08±0.74^{Bb}	78.50±0.31^{Cb}
	$FeSO_4$	87.83±0.30^{Ab}	85.75±0.70^{Ab}	83.25±0.58^{Bb}	79.41±0.47^{Cb}

Data are presented as mean±SEM (n=3), ab, Means with different superscripts within columns differ significantly ($p<0.05$). AB, Means with different superscripts within rows differ significantly ($p<0.05$).

Table 2: Effect of BCH-Iron complex on the color profile of HTST pasteurized milk during storage at 4 °C

Parameter/Days		1	3	5	7
L * value	control	86.71±0.050^{Aa}	86.68±0.033^{Ba}	86.51±0.046^{Ca}	86.31±0.103^{Da}
	BCH-Iron	82.37±0.024^{Ac}	82.45±0.023^{Bc}	82.63±0.056^{Cc}	82.64±0.042^{Db}
	$FeSO_4$	86.42±0.029^{Ab}	86.36±0.043^{Bb}	86.12±0.020^{Cb}	86.27±0.038^{Da}
a * value	control	-1.51±0.030^{Aa}	-1.42±0.021^{Ba}	-1.36±0.016^{Ca}	-1.28±0.020^{Da}
	BCH-Iron	1.21±0.015^{Ab}	1.18±0.007^{Bb}	1.09±0.026^{Cb}	1.03±0.027^{Db}
	$FeSO_4$	-1.67±0.015^{Ac}	-1.56±0.022^{Bc}	-1.44±0.029^{Cc}	-1.39±0.049^{Dc}
b * value	control	10.22±0.049^{Aa}	10.41±0.059^{Ba}	10.26±0.015^{Aa}	10.19±0.010^{Aa}
	BCH-Iron	13.35±0.021^{Ab}	13.37±0.019^{Ab}	13.35±0.021^{Ab}	13.51±0.035^{Bb}
	$FeSO_4$	10.24±0.013^{Ba}	10.23±0.034^{Ba}	10.32±0.049^{Ba}	10.15±0.054^{Aa}

Data are presented as mean±SEM (n=3), ab, Means with different superscripts within columns differ significantly ($p<0.05$). AB, Means with different superscripts within rows differ significantly ($p<0.05$).

Table 3: Effect of BCH-Iron complex on the physico-chemical properties of HTST pasteurized milk during storage at 4 °C

Parameter/days		1	3	5	7
Acidity (% LA)	Control	0.135±0.0008^{Aa}	0.141±0.0003^{Ba}	0.149±0.0005^{Ca}	0.157±0.0003^{Da}
	BCH-Iron	0.134±0.001^{Aa}	0.142±0.0003^{Ba}	0.152±0.0003^{Cb}	0.158±0.0005^{Da}
	$FeSO_4$	0.136±0.003^{Aa}	0.143±0.0003^{Ba}	0.154±0.0003^{Cb}	0.158±0.0003^{Da}
pH	Control	6.723±0.008^{Aa}	6.686±0.003^{Ba}	6.62±0.005^{Ca}	6.586±0.003^{Da}
	BCH-Iron	6.743±0.003^{Aa}	6.703±0.003^{Ba}	6.636±0.003^{Ca}	6.568±0.30^{Da}
	$FeSO_4$	6.688±0.006^{AB}	6.646±0.008^{Bb}	6.596±0.003^{Cb}	6.566±0.003^{Db}
Viscosity (cP)	Control	1.58±0.014^{Aa}	1.60±0.004^{Ba}	1.57±0.004^{Aa}	1.63±0.007^{Ca}
	BCH-Iron	1.58±0.014^{Ab}	1.61±0.004^{Bb}	1.59±0.020^{Ab}	1.63±0.004^{Cb}
	$FeSO_4$	1.59±0.012^{Ab}	1.61±0.004^{Bb}	1.59±0.012^{Ab}	1.63±0.004^{Cbc}
TBA (OD)	Control	0.053±0.0006^{Aa}	0.101±0.001^{Ba}	0.124±0.001^{Ca}	0.137±0.0005^{Da}
	BCH-Iron	0.073±0.0008^{Ab}	0.11±0.0009^{Bb}	0.142±0.0004^{Cb}	0.151±0.0004^{Db}
	$FeSO_4$	0.129±0.0004^{Ac}	0.165±0.003^{Bc}	0.193±0.002^{Cc}	0.226±0.003^{Dc}
FFA (μ equi/mL)	Control	1.25±0.012^{Aa}	1.58±0.12^{Ba}	1.78±0.07^{Ca}	1.86±0.02^{Da}
	BCH-Iron	1.23±0.003^{Aa}	1.62±0.02^{Bb}	1.82±0.12^{Cb}	1.90±0.009^{Db}
	$FeSO_4$	1.30±0.01^{Ab}	1.70±0.009^{Bc}	1.86±0.015^{Cc}	1.94±0.015^{Dc}

Data are presented as mean±SEM (n=3), ab, Means with different superscripts within columns differ significantly ($p<0.05$). AB, Means with different superscripts within rows differ significantly ($p<0.05$).

Table 4: Iron content of HTST pasteurized milk during storage at 4 °C

	HTST pasteurized milk		
Iron (ppm)	0.9±0.03^{B}	31.58±0.17^{A}	29.70±0.12^{A}

Data are presented as mean±SEM (n=3), AB, Means with different superscripts within column differ significantly ($p<0.05$).

Table 5: Effect of BCH-Iron complex on the microbiological properties of HTST pasteurized milk during storage at 4 °C

Parameter/Days		1	3	5	7
SPC (log cfu/mL)	Control	3.85±0.57^{Aa}	4.12±0.57^{Aa}	4.41±0.57^{Ba}	4.67±0.57^{Ba}
	BCH-Iron	3.85±0.57^{Aa}	4.14±0.57^{Aa}	4.41±0.57^{Ba}	4.67±0.57^{Ba}
	$FeSO_4$	3.85±0.57^{Aa}	4.13±0.57^{Aa}	4.41±0.57^{Ba}	4.67±0.57^{Ba}
Coliforms (log cfu/mL)	Control	NIL	NIL	NIL	NIL
	BCH-Iron	NIL	NIL	NIL	NIL
	$FeSO_4$	NIL	NIL	NIL	NIL

Yeast and Mold (log cfu/mL)	Control	NIL	NIL	NIL	NIL
	BCH-Iron	NIL	NIL	NIL	NIL
	$FeSO_4$	NIL	NIL	NIL	NIL

Data are presented as mean±SEM (n=3), ab, Means with different superscripts within columns differ significantly ($p<0.05$). AB, Means with different superscripts within rows differ significantly ($p<0.05$).

Conclusions
BCH-Iron complex having 1.4 mg/ml iron content was selected for fortification in HTST pasteurized milk and fortification was done at 30 ppm. Non-significant ($p>0.05$) difference of BCH-Iron complex fortification was observed while the storage significantly ($p>0.05$) affected the score of all sensory attributes on 7th day of storage. Acidity, pH, viscosity of the BCH-Iron complex HTST pasteurized milk was comparable to the control throughout the storage. BCH-Iron complex was promising in reduction of lipid oxidation and TBA value found significantly ($P<0.05$) lower than FeSO4 fortified sample. Colour profile of BCH-Iron complex was significantly ($p>0.05$) different compare to control.

Acknowledgement
The first author is thankful to the National Dairy Research Institute (ICAR), Karnal for the financial assistance in the form of Institutional Fellowship received during his M.Tech programme.

Reference
1. Abbasi S, Azari S. Efficiency of novel iron microencapsulation techniques: fortification of milk. International Journal of Food Science and Technology 2011; 46:1927-1933.
2. Abdulghani AH, Prakash S, Ali MY, Deeth HC. Sensory evaluation and storage stability of UHT milk fortified with iron, magnesium and zinc. Dairy Science and Technology 2015; 95:33-46.
3. AOAC International. Official methods of analysis of AOAC International. AOAC International, 2005.
4. Chen Z, Oldewage-Theron W. Food Fortification to Prevent and Control Iron Deficiency. African Journal of Food, Agriculture, Nutrition and Development 2002; 2:67-77.
5. Davies DT, White JCD. The stability of milk proteins to heat 1. Subjective measurement of heat stability of milk. Journal of Dairy Research 1966; 33:67-81.
6. El-Kholy AM, Osman M, Gouda A, Ghareeb WA. Fortification of yoghurt with iron. World Journal of Dairy and Food Sciences 2011; 6:159-165.
7. Fredlund K, Isaksson M, Rossander-Hulthen L, Almgren A, Sandberg AS. Absorption of zinc and retention of calcium: dose-dependent inhibition by phytate. Journal of Trace elements in Medicine and Biology. 2006; 20:49-57.
8. Gahruie HH, Eskandari MH, Mesbahi G, Hanifpour MA. Scientific and technical aspects of yogurt fortification: A review. Food Science and Human Wellness 2015; 4:1-8.
9. Gaucheron F. Iron fortification in dairy industry. Trends in Food Science and Technology 2000; 11:403-409.
10. Gu FL, Kim JM, Abbas S, Zhang XM, Xia SQ, Chen ZX. Structure and antioxidant activity of high molecular weight Maillard reaction products from casein–glucose. Food Chemistry. 2010; 120:505-511.
11. Hurrell R. How to ensure adequate iron absorption from iron fortified food. Nutrition Reviews. 2002; 60:7-15.
12. IS: 7768 Method for Sensory Evaluation of Milk. India: Bureau of Indian Standards, 1975.
13. IS: SP: 18 ISI Handbook of Food Analysis. Part. XI. Dairy Products. Indian Standards Institution, New Delhi, 1981.
14. Jairam BT, Vijayalakshmi BT, Balakrishnan CR, Nair KGS, Nair PG. Study on heat stability of cow's milk using an indigenous device. Indian Journal of Dairy Science, 1976.
15. King RL. Oxidation of milk fat globule membrane material. I. Thiobarbituric acid reaction as a measure of oxidized flavor in milk and model systems. Journal of Dairy Science. 1962; 45:1165-1171.
16. Mehansho H. Iron fortification technology development: new approaches. Journal of Nutrition. 2006; 136:1059-1063.
17. Peres JM, Bouhallab SD, Bureau F, Neuville D, Maubois JL, Devroede G. Mechanisms of absorption of caseinophosphopeptide bound iron. The Journal of Nutritional Biochemistry. 1999; 10:215-222.
18. Rosenthal I, Rosen B, Bernstein S. Effects of milk fortification with ascorbic acid and iron. Milchwissenschaft. 1993; 48:676-679.
19. Sachdeva B, Kaushik R, Arora S, Indumathi KP. Impact of fortification with iron salts and vitamin A on the physicochemical properties of laboratory pasteurized toned milk and bioaccessibility of the added nutrients. International Journal of Dairy Technology. 2015; 68:253-260.
20. Saini SPS, Jain SC, Bains GS. Effect of iron fortification on flavour of buffalo milk. Indian journal of dairy science, 1987.
21. Santillan-Urquiza E, Ruiz-Espinosa H, Angulo-Molina A, Velez-Ruiz JF, Mendez-Rojas MA. Applications of nanomaterials in functional fortified dairy products: benefits and implications for human health. In Nanotechnology in the agri-food industry nutrient delivery, 2017, 293-328.
22. Sugiarto M, Ye A, Taylor MW, Singh H. Milk protein-iron complexes: Inhibition of lipid oxidation in an emulsion. Dairy Science and Technology 2010; 90:119-119.
23. Taylor MJ, Richardson T. Antioxidant activity of skim milk: effect of heat and resultant sulfhydryl groups. Journal of Dairy Science 1980; 63:1783-1795.