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Microencapsulated iron for fortification in yoghurt

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Iron deficiency anaemia is a significant nutritional problem in south asian countries including India, Bangladesh and Pakistan than anywhere else in the world. Even though yogurt is an excellent source of calcium and protein, it contains very little iron. Using dairy foods as a vehicle for supplementing iron seems to be advantage as iron-fortified dairy foods have a relatively high iron bioavailability. Keeping this in view, a study was envisaged to formulate microencapsulated whey protein-chelated iron (Fe-wp) using ferrous sulphate that could be used in the development of iron fortified yoghurt to have highly bio available iron with no effect on nutritional value or sensory properties of the yoghurt. Influence of iron on survival of yoghurt culture, TBA values of yoghurt and sensory properties of yoghurt were tested by control, free iron and encapsulated iron fortification. Statistically no significant (P>0.05) difference was noticed in count of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* between control and different iron fortified yoghurt treatments on 0, 7, 14 and 21 days. During storage period, the count of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* significantly (P<0.05) decreased both in control and as well as in iron fortified yoghurt and thus the fortified iron did not affect the viability of yoghurt bacteria. The TBA values of unencapsulated iron fortified yoghurt was significantly (P<0.05) higher when compared to control and encapsulated iron fortified yoghurt. Significant (P<0.05) difference was observed in astringent and oxidized flavour at 0, 7, 14 and 21st day of storage between control and different treatments of yoghurt. In addition, significant (P<0.05) difference was observed in overall preference at 0, 7, 14 and 21st day of storage between control and different treatments of yoghurt and between different storage periods. It is demonstrated that microencapsulated whey protein chelated iron can be added up to a level of 80 mg per lit. of yoghurt without altering the accepted appearance and sensorial attributes.

Key Words : Microencapsulation, Ferrous sulphate, Dairy product, Yoghurt

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INTRODUCTION

Iron deficiency anaemia is still the most prevalent nutritional problem, which affects 30 per cent of the world's population. This deficiency causes more than half the maternal deaths in the world (Juneja *et al.*, 2004). Iron deficiency adversely affects the cognitive performance, behaviour, and physical growth of children, immune status, physical capacity and work performance of all age groups and increases perinatal risks for mothers and neonates (WHO, 2001). Iron deficiency anaemia affects 60 per cent of Asian women of reproductive age and 40 to 50 per cent of children enrolled in preschool and primary grades (Joseph, 2000). It is estimated that up to half of all anaemia is caused by dietary iron deficiency. Fortification of daily foods to obtain the recommended daily dietary allowances for iron (10- 15 mg for adults) is one of the most effective solutions (Bender and Bender, 1997). Dairy products are widely

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consumed, providing high quality proteins, vitamins and minerals except iron. Lack of iron in dairy products decreases the iron density of diets when the proportion of dairy products in the diets increases, so it is logical that fortifying dairy products with iron may increases dietary iron density of the people who consume large amounts of dairy products. Of late, among the dairy products, yoghurt has been gaining widespread consumer acceptance owing to its health giving attributes. It is an excellent source of calcium and protein but as is typical of all dairy products, contains very little iron. Therefore, dairy products are logical vehicles for iron fortification because they have high nutritive values, reach target population and are widely consumed. However, iron fortification is difficult in food processing due to potential oxidized off-flavours, colour changes, and metallic flavours, probably as a result of lipid prooxidation of milk fat. Hence, the ideal iron compound for food fortification should be one that supplies highly bio-available iron, does not affect the nutritional value or sensory properties of the food, should be stable during food processing, and of low cost, in order to be accessible for the whole population (Boccio *et al.*, 1998).The bioavailability of ferrous iron, especially ferrous sulfate, is high because the solubility of ferrous iron is higher than that of ferric iron. Ferrous iron is not very stable in solution and is easily oxidised to the insoluble ferric form and hither to several methods of stabilizing ferrous iron in solution have been investigated and Iron-protein complexes have shown high iron bioavailability similar to $FeSO_4$. Keeping the above technical constraints, the proposed investigation of Micro encapsulation of whey protein chelated iron and incorporation in the development of fortified yoghurt has been designed in such a way that it will definitely supply highly bioavailability iron with no effect on nutritional value or sensory properties of the yoghurt and will be stable during processing and storage and will be of low cost.

METHODOLOGY

Experimental design :

Different treatments of yoghurt were designed as detailed below :

PY	-	Control-without addition of iron
PFSY1	-	20 mg / l of un-encapsulated ferrous sulphate
PFSY2	-	40 mg / l of un-encapsulated ferrous sulphate
MFSY1	-	20 mg / l of encapsulated whey protein chelated ferrous sulphate
MFSY2	-	40 mg / l of encapsulated whey protein chelated ferrous sulphate
MFSY3	-	80 mg / l of encapsulated whey protein chelated ferrous sulphate
MFSY4	-	100 mg / l of encapsulated whey protein chelated ferrous sulphate

Microencapsulation of iron by emulsion method:

Whey protein chelated iron (Fe-Wp) was prepared by adding 8 g of ferrous sulphate into 100 ml of 20 per cent whey protein solution and heating to precipitate the complex. The precipitate was centrifuged at 8000G for 5 min: washed once with 0.25 per cent lactic acid solution and twice with deionised water. Microencapsulated whey protein chelated iron (MFe-Wp) was prepared by method of Azzam (2009). One part of Fe-Wp mixed with four parts sodium alginate solution (3 %). To one part of the mixture 10 ml was then added drop wise to 5 parts of sunflower oil 50 ml containing 0.1w/v tween 80 and stirred at 200 rpm by magnetic stirrer. Within 10 minutes a turbid emulsion was obtained. Calcium chloride 0.05M was added quickly to the beaker until the water oil emulsion was broken. Calcium alginate encapsulated beads containing Fe-Wp were formed within 10 min. The microcapsules were collected by gentle centrifugation (350 g for 10 min) and washed with distilled water using the same centrifugation conditions, and stored at 4°C until used.

Preparation of plain yoghurt and iron fortified yoghurt:

Plain yoghurt was prepared using fresh milk. Skim milk powder at the rate of 4 per cent (w/v) and sugar at the rate of 6 per cent (w/v) were added to it and homogenized at 2500 psi. The contents were mixed well and pasteurized at 85°C for 30 minutes, cooled to room temperature and inoculated with 2 per cent of yoghurt cultures containing *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Streptococcus salivarius* ssp. *thermophilus*. It was then mixed well and incubated at 42°C for 4 to 5 hours and finally stored at 5°C. In regard to the treatment yoghurt, the encapsulated iron beads / unencapsulated iron were added separately as given in the experimental design in the respective treatments to 1 lit. of mix. It was then mixed well and incubated at 42°C for 4 to 5 hours and finally stored at 4 to 5°C.

Preparation of iron fortified yoghurt:

Different lots of iron fortified yoghurt were prepared using fresh milk. Skim milk powder at the rate of 4 per cent (w/v) and sugar at the rate of 6 per cent (w/v) were added to it and homogenized at 2500 psi. The contents were mixed well and pasteurized at 85°C for 30 minutes, cooled to room temperature and inoculated with 2 per cent of yoghurt cultures containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*. Then encapsulated iron beads / unencapsulated iron were added separately as per the treatments to 1 lit. of mix. It was then mixed well and incubated at 42°C for 4 to 5 hours and finally stored at 4 to 5°C.

The prepared iron fortified yoghurt was subjected to organoleptic evaluation, TBA value and enumeration of count of yoghurt bacteria employing the procedure given by Kim *et al*.(2003) and the data obtained in all the experiments were analyzed statistically by applying one way and two way ANOVA (Snedecor and Cochran, 1994).

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Thiobarbituric acid values of microencapsulated iron fortified yoghurt (absorbance at 532 nm):

In regard TBA value, significantly higher values were observed in unencapsulated iron fortified yoghurt (PFSY2), when compared to control and capsulated iron fortified yoghurt (IFY) treatments (Table 1). The data indicated that oxidation process may be quicker in yoghurt samples containing unencapsulated iron than in those containing iron in encapsulated form. These findings were in accordance with the findings of Kim *et al*. (2003), who reported that TBA absorbance was significantly lower in encapsulated iron fortified yoghurts than the unencapsulated iron fortified yoghurts. Similarly, Jayalalitha *et al*. (2012) also observed that oxidation process was quicker in yoghurt samples containing unencapsulated iron than in those containing encapsulated iron. This increase in TBA values of unencapsulated iron fortified yoghurt may be due to interaction of added iron with casein resulting in iron–casein complexes and the presence of O_2, acts as a pro-oxidant, resulting in accelerated lipid oxidation in yoghurt. It can be opined that Micro encapsulation of iron lead to reduced rate of fat oxidation and increased fat stability, which facilitated a decreased TBA value as observed in encapsulated iron fortified yoghurt.

Effect of iron fortification on viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* in yoghurt (log_{10}cfu/ml) :

Table 2 shows that statistically no significant (P>0.05) difference was noticed in count of *Lactobacillus delbrueckii* ssp. *bulgaricus* between control and IFY treatments on day 0 to 21. It is also observed that there was a significant(P<0.05) decrease in *Lactobacillus delbrueckii ssp. bulgaricus* counts as the storage period advances towards 21 days. These findings concurred with the findings of Kim *et al*. (2003) who reported that the mean counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* for control and other groups of yoghurt did not differ significantly at 0 day, and also the mean counts in all groups showed a decreasing trend during 20 days of storage at 4°C. Fortification of yoghurt with different iron salts had no effect on the total lactic acid bacteria in all treatments when fresh and during cold storage El-

Table 1 : Thiobarbituric acid values of micro encapsulated iron fortified yoghurt (Absorbance at 532 nm)

Treatments	Duration			
	0 day	7 days	14 days	21 days
PY	0.0132^{Aa} ±0.02	0.0164^{Ba} ±0.03	0.0227^{Ca} ±0.09	0.0346^{Da} ±0.03
PFSY1	0.0133^{Aa} ±0.02	0.0167^{Ba} ±0.03	0.0242^{Ca} ±0.03	0.0350^{Da} ±0.01
PFSY2	0.0135^{Aa} ±0.01	0.0392^{Bb} ±0.02	0.0743^{Cc} ±0.09	0.0989^{Dc} ±0.02
MFSY1	0.0132^{Aa} ±0.02	0.0165^{Ba} ±0.03	0.0227^{Ca} ±0.09	0.0345^{Da} ±0.04
MFSY2	0.0132^{Aa} ±0.02	0.0166^{Ba} ±0.04	0.0231^{Ca} ±0.09	0.0347^{Da} ±0.03
MFSY3	0.0133^{Aa} ±0.05	0.0167^{Ba} ±0.03	0.0235^{Ca} ±0.08	0.0348^{Da} ±0.04
MFSY4	0.0135^{Aa} ±0.03	0.0167^{Ba} ±0.06	0.0241^{Ca} ±0.01	0.0348^{Da} ±0.04

@Average of six trials

Kholy *et al*. (2011). So iron fortification did not significantly (P>0.05) affect the growth and viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* both in the fresh yoghurt and during storage. The metabolic enzymatic activity of the yoghurt starter culture could be the reason for increase in the acidity and decrease in the pH, which could be responsible for decreasing the viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* as the storage period advances beyond a certain period.

Effect of iron fortification on *Streptococcus salivarius* ssp. *Thermophilus* viability in yoghurt (log_{10}cfu/ml):

Table 3 shows that statistically no significant (P>0.05) difference was noticed in count of *Streptococcus salivarius* ssp. *thermophilus* between control and IFY treatments. *Streptococcus salivarius* ssp. *thermophilus* counts were decreased significantly (P<0.05) as the storage period increased among control and IFY. These findings were in consistent with the findings of Kim *et al*. (2003) who reported that mean counts of *Streptococcus salivarius* ssp. *thermophilus* for control and other groups of yoghurt were not significantly different. Similarly, Cavallini and Rossi (2009) reported that viability of mixed starter culture containing *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* decreased as the storage time increased in iron and calcium fortified soy yoghurt. The reduction of *Streptococcus salivarius* ssp. *thermophilus* counts on storage may be due to low pH and high acidic condition prevailing in the yoghurt beyond a certain period during storage.

Effect of iron fortification on bitterness, metallic flavour and astringent flavour in yoghurt :

The bitterness values and metallic flavour values of encapsulated iron fortified yoghurt were similar to control, and the bitterness values and metallic flavour values were not significantly (P>0.05) increased during storage periods between control and encapsulated iron fortified yoghurt. These results were partly in accordance with the findings of Kwak *et al.*(2003). The astringent flavour values of encapsulated iron fortified yoghurt treatment MFSY3 and unencapsulated iron fortified yoghurt treatment MFSY1 were also similar to control. These astringent flavour values were significantly (P<0.05) increased during storage periods. These results were partly in agreement with the findings of Kwak *et al.* (2003).

Effect of iron fortification on oxidative flavour in yoghurt :

The oxidized flavour values of encapsulated iron

Table 2 : Effect of iron fortification on viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* in yoghurt (log_{10}cfu/ml)

Treatments	Duration			
	0 day	7 days	14 days	21 days
PY	$9.15^{Aa} \pm 0.02$	$8.83^{Ba} \pm 0.01$	$8.19^{Ca} \pm 0.01$	$7.66^{Da} \pm 0.01$
PFSY1	$9.13^{Aa} \pm 0.01$	$8.68^{Ba} \pm 0.01$	$8.10^{Ca} \pm 0.01$	$7.47^{Da} \pm 0.01$
PFSY2	$9.07^{Aa} \pm 0.01$	$8.62^{Ba} \pm 0.01$	$8.16^{Ca} \pm 0.01$	$7.41^{Da} \pm 0.01$
MFSY1	$9.19^{Aa} \pm 0.01$	$8.56^{Ba} \pm 0.02$	$8.19^{Ca} \pm 0.01$	$7.55^{Da} \pm 0.01$
MFSY2	$9.29^{Aa} \pm 0.01$	$8.63^{Ba} \pm 0.01$	$8.20^{Ca} \pm 0.01$	$7.57^{Da} \pm 0.02$
MFSY3	$9.20^{Aa} \pm 0.01$	$8.95^{Ba} \pm 0.01$	$8.29^{Ca} \pm 0.01$	$7.71^{Da} \pm 0.01$
MFSY4	$9.19^{Aa} \pm 0.01$	$8.63^{Ba} \pm 0.01$	$8.19^{Ca} \pm 0.01$	$7.55^{Da} \pm 0.01$

@Average of six trials

Table 3 : Effect of iron fortification on *Streptococcus salivarius ssp. thermophilus* viability in yoghurt (log_{10}cfu/ml)

Treatments	Duration			
	0 day	7 days	14 days	21 days
PY	$8.93^{Aa} \pm 0.02$	$8.43^{Ba} \pm 0.01$	$7.82^{Ca} \pm 0.01$	$7.26^{Da} \pm 0.01$
PFSY1	$8.73^{Aa} \pm 0.01$	$8.11^{Ba} \pm 0.01$	$7.680^{Ca} \pm 0.01$	$7.17^{Da} \pm 0.01$
PFSY2	$8.72^{Aa} \pm 0.01$	$8.18^{Ba} \pm 0.01$	$7.56^{Ca} \pm 0.01$	$7.10^{Da} \pm 0.01$
MFSY1	$8.79^{Aa} \pm 0.01$	$8.26^{Ba} \pm 0.02$	$7.79^{Ca} \pm 0.01$	$7.15^{Da} \pm 0.01$
MFSY2	$8.83^{Aa} \pm 0.01$	$8.13^{Ba} \pm 0.01$	$7.60^{Ca} \pm 0.01$	$7.22^{Da} \pm 0.02$
MFSY3	$8.85^{Aa} \pm 0.01$	$8.35^{Ba} \pm 0.01$	$7.78^{Ca} \pm 0.01$	$7.24^{Da} \pm 0.01$
MFSY4	$8.81^{Aa} \pm 0.01$	$8.33^{Ba} \pm 0.01$	$7.79^{Ca} \pm 0.01$	$7.23^{Da} \pm 0.01$

@Average of six trials

fortified yoghurt treatment MFSY3 and unencapsulated iron fortified yoghurt treatment MFSY1 were similar to control. These oxidized flavour values were significantly ($P<0.05$) increased during storage between control and Iron Fortified Yoghurt treatments. Gaucheron (2000) reported that Micro encapsulation techniques can be used to avoid oxidized, metallic flavours and colour changes during fortification with iron. This is supported by the findings of Jayalalitha *et al*. (2012), who concluded that encapsulation treatment for iron will give the good sensory quality by avoiding the oxidized flavour in iron fortified yoghurt.

Effect of iron fortification on overall preference of yoghurt :

On sensory evaluation, all the panelists preferred control yoghurt and MFSY3 over other treatments and in that order of preference. This indicated that iron can be fortified only up to 20mg per litre in unencapsulated form, while in the form of microencapsulated iron it can be incorporated up to 80 mg per litre of yoghurt using ferrous sulfate without affecting the accepted appearance, sensorial and textural attributes of yoghurt.

It is concluded that fortified iron did not affect the viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* in yoghurt. The TBA values of unencapsulated iron fortified yoghurt was significantly higher when compared to control and capsulated iron fortified yoghurt. In conclusion, the present work indicated that microencapsulated whey protein chelated iron can be incorporated up to a level of 80 mg per litre of yoghurt without altering the accepted appearance and taste. This study concludes that iron fortification does not affect the viability of probiotic yoghurt bacteria and encapsulation treatment for iron will give the good sensory quality by avoiding the oxidized flaovur in iron fortified yoghurt, which can contribute to alleviating iron deficiency.

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