Original Research Article

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Culture Viability, TBA Values and Sensorial Attributes of Yoghurt Fortified with Microencapsulated Whey Protein-Chelated Iron

A. Elango¹*, R. Subash², K.A. Doraisamy³ and N. Karthikeyan⁴

¹Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, Tamil Nadu, India
²Bargur Cattle Research Station, Bargur, Erode, Tamil Nadu, India
³Veterinary College and Research Institute, Namakkal-637 002, Tamil Nadu, India
⁴Department of Livestock Products Technology (Dairy Science), Veterinary College and Research Institute, Namakkal-637 002, Tamil Nadu, India

*Corresponding author

A B S T R A C T

Using dairy foods as a vehicle for supplementing iron seems to be advantage as iron-fortified dairy foods have a relatively high iron bioavailability. In this regard, a study was designed to formulate microencapsulated whey protein-chelated iron (Fe-wp) using ferrous sulphate that could be used to fortify yoghurt and to determine the TBA values and some important sensorial attributes of the developed 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 litre of yoghurt without altering the accepted appearance and sensorial attributes.

Keywords
Yoghurt, Iron fortification, Microencapsulation, Culture survival, TBA values, Sensorial attributes.

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Introduction

Iron deficiency leads to anemia, altered mental development, decreased immunity, impaired cognitive scores. Iron deficiency anaemia is still the most prevalent nutritional problem, which affects 30 % of the world’s population. In India, 79% of children between 6 and 35 months and women between 15 and 49 years of age are anaemic; inadequate intake of iron and consumption of foods low in bioavailable iron are identified as the major
cause of iron deficiency anaemia (Tripathi and Platel, 2011)

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 % of Asian women of reproductive age and 40 to 50 % 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 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-flavors, color changes, and metallic flavors, probably as a result of lipid prooxidation of milk fat. Hence, the ideal iron compound for food fortification should be one that supplies highly bioavailable 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 microencapsulation 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.

Materials and Methods

Experimental design

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 per cent). 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 per the treatments to 1 litre 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).

Results and Discussion

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 mean (± SE) TBA of control and iron fortified yoghurt at 0, 7, 14 and 21 days of storage period are shown in Table 1. The TBA values of control and iron fortified yoghurt ranged from 0.0132±0.02 to 0.0989±0.02. Statistically significant (P<0.05) difference was noticed in TBA values between control and iron fortified yoghurts. During storage of yoghurt there was significant (P<0.05) increase in TBA was also observed between control and iron fortified yoghurt.

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

Table 2 shows the mean (± SE) values of count of Lactobacillus delbrueckii ssp. bulgaricus between control and iron fortified
yoghurt treatments ranged from 9.29 ± 0.01 to 7.41 ± 0.01 during storage period of 0, 7, 14 and 21 days at 5°C. Statistically no significant (P>0.05) difference was noticed in count of Lactobacillus delbrueckii ssp. bulgaricus between control and different iron fortified yoghurt (IFY) treatments on day 0. As the storage period advances to 21 days there was significant reduction in the count of Lactobacillus delbrueckii ssp. bulgaricus.

**Effect of iron fortification on Streptococcus salivarius ssp. thermophilus viability in yoghurt (log₁₀cfu/ml)**

Table 3 shows the mean (± SE) values of count of Streptococcus salivarius ssp. thermophilus between control and different IFY treatments ranged from 8.93 ± 0.02 to 7.10 ± 0.01 during storage period of 0, 7, 14 and 21 days at 5°C. Statistically no significant (P>0.05) difference was noticed in count of Streptococcus salivarius ssp. thermophilus between control and IFY treatments.

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

Table 4 shows the mean (± SE) values of bitterness between control and IFY treatments during storage period of 0, 7, 14 and 21 days at 5°C. The bitterness scores ranged from 1.05 ± 0.17 to 7.40 ± 0.12 between control and IFY treatments up to 21 days of storage at 5°C. No significant (P>0.05) difference was noticed between control and IFY treatments at 0 day, whereas significant (P<0.05) difference was observed at 7, 14 and 21st day of storage.

Table 5 shows the mean (± SE) values of metallic flavour between control and IFY treatments during storage period of 0, 7, 14 and 21 days at 5°C. The metallic flavour scores ranged from 1.04 ± 0.17 to 7.49 ± 0.12 between control and IFY treatments during 21 days of storage period at 5°C. No significant (P>0.05) difference was noticed between control and IFY treatments at 0 day, whereas significant (P<0.05) difference was observed at 7, 14 and 21st day of storage between control and IFY treatments.

Table 6 shows the mean (± SE) values of astringent flavour scores between control and IFY treatments during storage period of 0, 7, 14 and 21 days at 5°C. The astringent flavour scores ranged from 1.30 ± 0.06 to 7.30 ± 0.15 between control and IFY treatments during 21 days of storage period at 5°C. Significant (P<0.05) difference was observed in astringent flavour at 0, 7, 14 and 21st day of storage between control and IFY treatments.

**Different treatments of yoghurt were designed as detailed below**

| PY       | Control-without addition of iron |
|----------|----------------------------------|
| PFSY1    | 20 mg / litre of un-encapsulated ferrous sulphate |
| PFSY2    | 40 mg / litre of un-encapsulated ferrous sulphate |
| MFSY1    | 20 mg / litre of encapsulated whey protein chelated ferrous sulphate |
| MFSY2    | 40 mg / litre of encapsulated whey protein chelated ferrous sulphate |
| MFSY3    | 80 mg / litre of encapsulated whey protein chelated ferrous sulphate |
| MFSY4    | 100 mg / litre of encapsulated whey protein chelated ferrous sulphate |
Table 1. Thiobarbituric acid values of microencapsulated iron fortified yoghurt (Absorbance at 532 nm)

| Treatment | 0 day | 7 days | 14 days | 21 days |
|-----------|-------|--------|---------|---------|
| PY        | 0.0132±0.02 | 0.0164±0.03 | 0.0227±0.09 | 0.0346±0.03 |
| PFSY1     | 0.0133±0.02 | 0.0167±0.03 | 0.0242±0.03 | 0.0350±0.01 |
| PFSY2     | 0.0135±0.01 | 0.0392±0.02 | 0.0743±0.09 | 0.0989±0.02 |
| MFSY1     | 0.0132±0.02 | 0.0165±0.03 | 0.0227±0.09 | 0.0345±0.04 |
| MFSY2     | 0.0132±0.02 | 0.0166±0.04 | 0.0231±0.09 | 0.0347±0.03 |
| MFSY3     | 0.0133±0.05 | 0.0167±0.03 | 0.0235±0.08 | 0.0348±0.04 |
| MFSY4     | 0.0135±0.03 | 0.0167±0.06 | 0.0241±0.01 | 0.0348±0.04 |

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

| Treatment | 0 day | 7 days | 14 days | 21 days |
|-----------|-------|--------|---------|---------|
| PY        | 9.15±0.02 | 8.83±0.01 | 8.19±0.01 | 7.66±0.01 |
| PFSY1     | 9.13±0.01 | 8.66±0.01 | 8.10±0.01 | 7.47±0.01 |
| PFSY2     | 9.07±0.01 | 8.63±0.01 | 8.16±0.01 | 7.41±0.01 |
| MFSY1     | 9.19±0.01 | 8.56±0.02 | 8.19±0.01 | 7.55±0.01 |
| MFSY2     | 9.29±0.01 | 8.63±0.01 | 8.20±0.01 | 7.57±0.02 |
| MFSY3     | 9.20±0.01 | 8.95±0.01 | 8.29±0.01 | 7.71±0.01 |
| MFSY4     | 9.19±0.01 | 8.63±0.01 | 8.19±0.01 | 7.55±0.01 |

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

| Treatment | 0 day | 7 days | 14 days | 21 days |
|-----------|-------|--------|---------|---------|
| PY        | 8.93±0.02 | 8.43±0.01 | 7.82±0.01 | 7.26±0.01 |
| PFSY1     | 8.73±0.01 | 8.11±0.01 | 7.68±0.01 | 7.17±0.01 |
| PFSY2     | 8.72±0.01 | 8.18±0.01 | 7.56±0.01 | 7.10±0.01 |
| MFSY1     | 8.79±0.01 | 8.26±0.02 | 7.78±0.01 | 7.15±0.01 |
| MFSY2     | 8.83±0.01 | 8.13±0.01 | 7.60±0.01 | 7.22±0.02 |
| MFSY3     | 8.85±0.01 | 8.35±0.01 | 7.78±0.01 | 7.24±0.01 |
| MFSY4     | 8.81±0.01 | 8.33±0.01 | 7.79±0.01 | 7.23±0.01 |

Table 4. Effect of iron fortification on bitterness in yoghurt

| Treatment | 0 day | 7 days | 14 days | 21 days |
|-----------|-------|--------|---------|---------|
| PY        | 1.05±0.17 | 1.10±0.09 | 1.15±0.09 | 1.17±0.10 |
| PFSY1     | 1.23±0.11 | 1.46±0.15 | 1.50±0.12 | 1.63±0.09 |
| PFSY2     | 1.60±0.13 | 3.80±0.14 | 5.20±0.11 | 7.40±0.12 |
| MFSY1     | 1.11±0.20 | 1.20±0.15 | 1.33±0.17 | 1.38±0.18 |
| MFSY2     | 1.12±0.19 | 1.21±0.15 | 1.33±0.17 | 1.39±0.18 |
| MFSY3     | 1.13±0.20 | 1.22±0.15 | 1.33±0.17 | 1.39±0.19 |
| MFSY4     | 1.11±1.19 | 1.19±0.14 | 1.31±0.16 | 1.37±0.18 |
Table 5: Effect of iron fortification on metallic flavour in yoghurt

| Treatment | Duration |
|-----------|----------|
|           | 0 day    | 7 days | 14 days | 21 days |
| PY        | 1.04 ±0.17 | 1.11 ±0.09 | 1.14 ±0.09 | 1.16 ±0.10 |
| PFSY1     | 1.24 ±0.11 | 1.45 ±0.15 | 1.50 ±0.12 | 1.62 ±0.09 |
| PFSY2     | 1.60 ±0.13 | 3.82 ±0.14 | 5.23 ±0.11 | 7.49 ±0.12 |
| MFSY1     | 1.11 ±0.20 | 1.20 ±0.15 | 1.33 ±0.17 | 1.38 ±0.18 |
| MFSY2     | 1.12 ±0.19 | 1.21 ±0.15 | 1.32 ±0.17 | 1.39 ±0.18 |
| MFSY3     | 1.13 ±0.20 | 1.22 ±0.15 | 1.33 ±0.17 | 1.39 ±0.19 |
| MFSY4     | 1.11 ±0.19 | 1.19 ±0.14 | 1.31 ±0.16 | 1.37 ±0.18 |

Table 6: Effect of iron fortification on astringency in yoghurt

| Treatment | Duration |
|-----------|----------|
|           | 0 day    | 7 days | 14 days | 21 days |
| PY        | 1.30 ±0.06 | 1.46 ±0.07 | 1.66 ±0.09 | 1.98 ±0.07 |
| PFSY1     | 1.50 ±0.09 | 1.76 ±0.09 | 2.21 ±0.06 | 2.50 ±0.08 |
| PFSY2     | 2.60 ±0.06 | 3.83 ±0.16 | 5.50 ±0.08 | 7.30 ±0.14 |
| MFSY1     | 1.30 ±0.12 | 1.50 ±0.13 | 1.80 ±0.06 | 2.20 ±0.09 |
| MFSY2     | 1.41 ±0.09 | 1.51 ±0.14 | 1.82 ±0.05 | 2.21 ±0.09 |
| MFSY3     | 1.42 ±0.09 | 1.52 ±0.13 | 1.83 ±0.06 | 2.28 ±0.12 |
| MFSY4     | 1.30 ±0.14 | 1.50 ±0.12 | 1.80 ±0.06 | 2.18 ±0.09 |

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

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 O₂ acts as a pro-oxidant, resulting in accelerated lipid oxidation in yoghurt. It can be opined that microencapsulation 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 (log₁₀ cfu/ml)

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-Kholy (2011). So iron fortification did not significantly (P>0.05) affect the growth and viability of *Lactobacillus delbrueckii* sp. *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<sub>10</sub>cfu/ml)**

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 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 microencapsulation 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.

Upon evaluation of the prepared yoghurt, it is concluded that that microencapsulated whey protein chelated iron can be incorporated up to a level of 80 mg per litre of yoghurt without affecting the accepted appearance and taste and the viability of probiotic yoghurt bacteria, which will definitely contribute in alleviating issues related to iron deficiency.

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