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Original Article

THE VIABILITY OF YOGHURT PROBIOTIC CULTURE IN MICROENCAPSULATED IRON FORTIFIED YOGHURT

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Abstract:** A study was designed to develop microencapsulated whey protein-chelated iron (Fe-wp) using ferrous sulphate as the iron source that could be used in the development of iron fortified 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 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. The present study demonstrated that microencapsulated whey protein chelated iron can be added up to a level of 80 mg per litre of yoghurt using ferrous sulphate without affecting the viability of yoghurt probiotic culture.

Key words: Yoghurt, Microencapsulation, Whey protein chelated iron

INTRODUCTION

Yoghurt is widely consumed throughout the world for its sensory and nutritional benefits and is made from milk with high solid content, a lactic culture and sugar and can be enriched with milk powder, proteins, vitamins, minerals and fruits. Yoghurt is a product obtained by the lactic fermentation of whole, skimmed or standardized milk by action of *Lactobacillus delbrueckii*ssp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, and can be accompanied by other lactic bacteria which, for their part, contribute to the characteristics of the final product [1].

Iron deficiency anemia is still the most prevalent nutritional problem, which affect 30 % of the world's population. This deficiency causes more than half the maternal deaths in the world [2]. 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 [3]. Iron deficiency anemia affects 60

% of Asian women of reproductive age and 40 to 50 % of children enrolled in preschool and primary grades [4]. It is estimated that up to half of all anemia is caused by dietary iron deficiency. Fortification of dairy foods to obtain the recommended daily dietary allowances for iron (10- 15 mg for adults) is one of the most effective solutions [5]. Therefore, 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 [6]. Yoghurt is an excellent source of calcium and protein but as it 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.

In this study ferrous sulfate which is completely dissolved in water and thus provide very high bioavailable iron was selected for fortification of yoghurt. Conventionally, methods for increasing iron content in foods have been used to directly add iron to foods. However, in order to alleviate problems of conventional methods like disagreeable smell due to oxidation of fat in milk, discoloration and precipitation of iron, an attempt has been made to microencapsulate iron and apply it to yoghurt, infant formula, milk powder, cheese and foods [7].

Microencapsulation, which shows potential as a carrier of enzymes in the food industry, could be a good vehicle for the addition of iron to milk [8]. Microencapsulation is a technology of packaging solids, liquids or gaseous materials in miniature sealed capsules that can release their contents at controlled rates under the influences of specific conditions. Keeping the above 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 bio-available iron with no effect on nutritional value or sensory properties of yoghurt, will be stable during processing as well as storage and will be of low cost.

MATERIALS AND METHODS

Experimental design: 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

Emulsion method of microencapsulation of iron: 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 [9]. 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 4ÚC until used.

Procedure for the preparation of plain 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 85ÚC 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 42ÚC for 4 to 5 hours and finally stored at 5ÚC.

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 85ÚC 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 litre of mix. It was then mixed well and incubated at 42^0C for 4 to 5 hours and finally stored at 4 to 5^0C.

Enumeration of yoghurt bacteria in yoghurt [10]: Lactic acid bacteria were determined from the colony counts on specific lactic agar: MRS agar (pH 5.4) for *Lactobacillus delbrueckiisubsp. bulgaricus* and M17 agar for *Streptococcusalivarius ssp. thermophilus*. One gram yoghurt samples stored for 0, 7, 14 and 21 day were diluted with 9 ml of sterile peptone and water diluent. Subsequent serial dilutions of each sample was performed. From the suitable dilution 1 ml was transferred into sterile petri plates in duplicates. Pre-melted MRS and M17 agar (20-30 ml) were poured into the plate and mixed well with the contents. After solidification the plates were incubated at 41°C for 48 h.

Estimation of TBA value in iron fortified yoghurt [10]: The reagent for TBA test was prepared immediately before use by mixing equal volumes of freshly prepared 0.025M TBA, which was neutralized with NaOH and 2M H_3PO/2 mcitric acid. Reactions of TBA test were started by pipetting 5.0 ml of milk containing iron capsulated or uncapsulated into a glass centrifuge tube and mixed thoroughly with 2.5 ml TBA reagent. The mixture was heated immediately in a boiling water bath for exactly 10 min, and cooled on ice. Ten ml of cyclohexanone and 1 ml of 4M Ammonium sulfate were added and centrifuged at 2490×G for 5 min at room temperature. The orange-red cyclohexanone supernatant was decanted and its absorbance at 532 nm was measured spectrophotometrically in a 1-cm light path. All measurements were run in triplicate.

Organoleptic evaluation [10]: Organoleptic evaluation was carried out by untrained panel of judges comprising five members. The intensity of taste aspects (bitterness, oxidative flavor and metallic flavor) were scored on a nine-point scale (1= none, 3= slight, 5= moderate, 7= strong and 9= very strong). Overall preference were scored on a nine-point scale (1= dislike extremely, 3= dislike moderate, 5= neither like or dislike, 7= like moderate and 9= like extremely). All the samples were appropriately coded before subjected for sensory evaluation.

Statistical analysis: The data obtained in all the experiments were analyzed statistically by applying one way and two way ANOVA [11].

RESULTS AND DISCUSSION

Effect of iron fortification on viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* in yoghurt (log_{10}cfu/ml): Table 1 shows that statistically insignificant (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. [10] 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 4ÚC. 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 [12]. 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 2 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. [10] who reported that mean counts of

Table 1: Effect of iron fortification on viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* in yoghurt ($\log_{10}$cfu/ml). Average of six trials. Different superscripts in uppercase in a row differ significantly and Different superscripts in lowercase in a column differ significantly.

Treatment	Duration			
	0 day	7 days	14 days	21 days
PY	$9.15^{Aa}\pm 0.02$	$8.83^{Ba}\pm0.01$	$8.19^{Ca}\pm0.01$	$7.66^{Da}\pm0.01$
PFSY1	$9.13^{Aa}\pm0.01$	$8.68^{Ba}\pm0.01$	$8.10^{Ca}\pm0.01$	$7.47^{Da}\pm0.01$
PFSY2	$9.07^{Aa}\pm0.01$	$8.62^{Ba}\pm0.01$	$8.16^{Ca}\pm0.01$	$7.41^{Da}\pm0.01$
MFSY1	$9.19^{Aa}\pm0.01$	$8.56^{Ba}\pm0.02$	$8.19^{Ca}\pm0.01$	$7.55^{Da}\pm0.01$
MFSY2	$9.29^{Aa}\pm0.01$	$8.63^{Ba}\pm0.01$	$8.20^{Ca}\pm0.01$	$7.57^{Da}\pm0.02$
MFSY3	$9.20^{Aa}\pm0.01$	$8.95^{Ba}\pm0.01$	$8.29^{Ca}\pm0.01$	$7.71^{Da}\pm0.01$
MFSY4	$9.19^{Aa}\pm0.01$	$8.63^{Ba}\pm0.01$	$8.19^{Ca}\pm0.01$	$7.55^{Da}\pm0.01$

Table 2: Effect of iron fortification on *Streptococcus salivarius ssp. thermophilus* viability in yoghurt ($\log_{10}$cfu/ml). Average of six trials. Different superscripts in uppercase in a row differ significantly and Different superscripts in lowercase in a column differ significantly.

Treatment	Duration			
	0 day	7 days	14 days	21 days
PY	$8.93^{Aa}\pm 0.02$	$8.43^{Ba}\pm0.01$	$7.82^{Ca}\pm0.01$	$7.26^{Da}\pm0.01$
PFSY1	$8.73^{Aa}\pm0.01$	$8.11^{Ba}\pm0.01$	$7.680^{Ca}\pm0.01$	$7.17^{Da}\pm0.01$
PFSY2	$8.72^{Aa}\pm0.01$	$8.18^{Ba}\pm0.01$	$7.56^{Ca}\pm0.01$	$7.10^{Da}\pm0.01$
MFSY1	$8.79^{Aa}\pm0.01$	$8.26^{Ba}\pm0.02$	$7.79^{Ca}\pm0.01$	$7.15^{Da}\pm0.01$
MFSY2	$8.83^{Aa}\pm0.01$	$8.13^{Ba}\pm0.01$	$7.60^{Ca}\pm0.01$	$7.22^{Da}\pm0.02$
MFSY3	$8.85^{Aa}\pm0.01$	$8.35^{Ba}\pm0.01$	$7.78^{Ca}\pm0.01$	$7.24^{Da}\pm0.01$
MFSY4	$8.81^{Aa}\pm0.01$	$8.33^{Ba}\pm0.01$	$7.79^{Ca}\pm0.01$	$7.23^{Da}\pm0.01$

Table 3: Thiobarbituric acid values of microencapsulated iron fortified yoghurt (Absorbance at 532 nm). Average of six trials. Different superscripts in upper case in a row differ significantly and Different superscripts in lowercase in a column differ significantly.

Treatment	Duration			
	0 day	7 days	14 days	21 days
PY	$0.0132^{Aa}\pm0.02$	$0.0164^{Ba}\pm0.03$	$0.0227^{Ca}\pm0.09$	$0.0346^{Da}\pm0.03$
PFSY1	$0.0133^{Aa}\pm0.02$	$0.0167^{Ba}\pm0.03$	$0.0242^{Ca}\pm0.03$	$0.0350^{Da}\pm0.01$
PFSY2	$0.0135^{Aa}\pm0.01$	$0.0392^{Bb}\pm0.02$	$0.0743^{Cc}\pm0.09$	$0.0989^{Dc}\pm0.02$
MFSY1	$0.0132^{Aa}\pm0.02$	$0.0165^{Ba}\pm0.03$	$0.0227^{Ca}\pm0.09$	$0.0345^{Da}\pm0.04$
MFSY2	$0.0132^{Aa}\pm0.02$	$0.0166^{Ba}\pm0.04$	$0.0231^{Ca}\pm0.09$	$0.0347^{Da}\pm0.03$
MFSY3	$0.0133^{Aa}\pm0.05$	$0.0167^{Ba}\pm0.03$	$0.0235^{Ca}\pm0.08$	$0.0348^{Da}\pm0.04$
MFSY4	$0.0135^{Aa}\pm0.03$	$0.0167^{Ba}\pm0.06$	$0.0241^{Ca}\pm0.01$	$0.0348^{Da}\pm0.04$

Streptococcus salivarius ssp. *thermophilus* for control and other groups of yoghurt were not significantly different. Similarly, Cavallini and Rossi [13] found 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.

Thiobarbituric acid values of microencapsulated iron fortified yoghurt (absorbance at 532 nm): Table 3 shows that significantly higher TBA values were observed in unencapsulated iron fortified yoghurt (PFSY2), when compared to control and capsulated iron fortified yoghurt (IFY) treatments. 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. [10], who reported that TBA absorbance was significantly lower in encapsulated iron fortified yoghurts than the unencapsulated iron fortified yoghurts. Similarly, Jayalalitha et al. [14] 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 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 bitterness and metallic 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. [15].

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 PFSY1 were similar to control. These oxidized flavour values were significantly (P<0.05) increased during storage between control and iron fortified yoghurt treatments. Gaucheron [16] 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. [14], 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 panellists preferred control yoghurt, MFSY3 and PFSY1 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 upto 80 mg per litre of yoghurt using ferrous sulphate without affecting the accepted appearance, sensorial and textural attributes of yoghurt.

CONCLUSION

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. The present work indicated that microencapsulated whey protein chelated iron can be incorporated up to a level of 80 mg per litre of yoghurt using ferrous sulphate 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 flavour in iron fortified yoghurt, which can contribute to alleviating iron deficiency.

REFERENCES

[1] Brasil: Ministerio da Agricultura, Pecuaria e AbasbecimentoInstrucao Normativa n 46 de 23 de Outubro de. downloaded from www.agricultura.-gov.br (2007).

[2] Juneja, L.R., Sakaguchi, N., Yamaguchi, R. and Nanbu, H.: Iron fortification of dairy products: A Novel approach. In: *Handbook of Functional Dairy Products* (Short, C. and Brrien, J.O. eds), CRC Press, London (2004).

[3] World Health Organisation.: Iron deficiency anemia : Assessment, Prevention and Control. A guide for programme managers, Geneva. Disponivelem: http://whplibdoc.who.int/hp/2001/who_NHD_01.-3.pdf.Acessoem: 27 May 2013 (2001).

[4] Joseph, M.H.: *Why countries and companies should invest to eliminate micronutrient malnutrition*, Manila Forum : Strategies to fortify essential foods in Asia and the Pacific nutrition and development series, Asian development, International Life Sciences Institute, and Micronutrient Initiative, 32-41 (2000).

[5] Bender, D.A. and Bender, A.E.: *Nutrition a Reference Handbook*. Oxford Univ. Press, pp 394 (1997).

[6] Boccio, J.R., Zubillaga, M.B., Caro, R.A., Lysionek, A., Gotelli, C.A., Gotelli, M.J. and Weill, R.: Biol. Trace Elem. Res., 62: 65-73 (1998).

[7] Kwak, H.S. and Yang, K.M.: *Method for preparing microcapsule containing soluble iron using fatty acid ester*. U.S. Patent. 6402997 B1 (2002).

[8] Jackson, L.S. and Lee, K.: J. Food Sci., 56(4): 1047-1050 (1991).

[9] Azzam, M. A.: J. Dairy. Sci., 37: 55-63 (2009).

[10] Kim, S.J., Ahn, J. and Kwak, H.S.: Asian Austral. J. Anim. Sci., 16: 581-587 (2003).

[11] Snedecor, G.W. and Cochran, W.G.: *Statistical methods*. Eighth edition, IOWA State University Press, USA (1994).

[12] El-Kholy, A. M., Osman, M., Gouda, A. and Ghareeb, W.A.: World J. Dairy Food Sci., 6: 159-165 (2011).

[13] Cavallini, D.C.U. and Rossi, E.A.: Alim. Nutr., 20(1): 7-13 (2009).

[14] Jayalalitha, V., Balasundaram, V., Palanidorai, B. and Naresh Kumar, C.: Int. J. Agric., 2(2): 80-84 (2012).

[15] Kwak, H.S., Yang, K.M.andAhn, J.: J. Agric. Food. Chem., 51(26): 7770-7774 (2003).

[16] Gaucheron, F.: Iron fortification in dairy industry. Trends Food Sci. Tech., 11: 403-409 (2000).