

Efficiency of Novel Iron Microencapsulation Techniques: Fortification of Milk

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INTRODUCTION AND OBJECTIVES

Iron is an essential trace element in animal and human diets. Lack of this trace element is the commonest nutritional deficiency around the world that causes iron deficiency anemia. In general, three approaches are recommended for amending nutritional iron deficiency, in an individual or combined forms: a) dietary modification and diversification to improve nutritional value and iron bioavailability, b) supplementation and c) fortification (the addition of micronutrients to the processed foods). Fortification is widely considered as a practical and cost-effective long-term solution (Zimmermann and Hurrell, 2007). Milk fortification with iron is not an easy task because it may cause metallic taste, unacceptable flavor as a result of the oxidation or rancidity of fats, undesirable color changes resulting from interaction with anthocyanins, flavonoids, and tannins, and degradation of vitamins and minerals. It is therefore proposed that iron salts should be microencapsulated to prevent these negative effects (Xia and Xu, 2005).

Despite all attempts had been made, there is lack of information about the comparison of abovementioned methods together and with commercially available microencapsulated irons, their efficiencies, and their sensory evaluations. Therefore, the present study concentrated on the microencapsulation of various iron salts with liposome and F.A.E. methods, investigation of the effects of major variables on their encapsulation efficiency, physical stability, as well as sensory properties of pasteurized milk during storage at refrigerated circumstances.

MATERIALS AND METHODS

Microencapsulation by liposome and F.A.E. methods

For liposome method, 1.14 g lecithin and 0.06 g cholesterol was initially dissolved in 30 ml diethyl ether. Then, the organic phase was mixed with 10 mL of citric acid-sodium phosphate buffer solution [pH=6.8, containing iron salt: ascorbic (15:1 w/w)]. At this stage, various weight ratios of iron ion to lipid phase (0.04 and 0.1) were used. For emulsification purposes, the mixture sonicated (UP 400S, Dr.Hielscher, Germany) for 7 min at 270 W. Afterwards, the organic solvent was removed and a gel was formed. Upon persistent evaporation, the gel was broken and the remaining aqueous phase was added and the rest of ether was evacuated. Then, the liposomes with various concentrations of iron and Tween 80 were stored at 4 °C prior to be used (Xia and Xu, 2005).

In terms of F.E.A method, a mixture containing 50 mL of distilled water and 5 g of PGMS was heated (for 20 min

at 55 °C) and stirred at 1200 rpm for 2 min. In the next step, iron salt and ascorbic acid was added into the previously made solution and whole mixture was stirred at 1200 rpm for 1 min. Subsequently, the coat:core emulsions (at 45 °C) were nebulized using an airless sprayer (W180P, Wagner Spray Tech. Co., Markdorf, Germany) into a Tween 60 solution. Then, the chilled Tween 60 solution was centrifuged in order to separate the microcapsules. After that, the microcapsules with different weight ratios of coat: core materials were collected and stored at 4 °C prior to be used (Kwak *et al.*, 2003, 2001).

Fortification of milk with microencapsulated irons For this purpose, four types of iron (including iron microencapsulated by liposome and F.A.E. methods, commercial microcapsules and plain or non-encapsulated iron) at 3 various levels were added to the pasteurized milk where the final iron concentration of iron was about 7, 14, and 21 mg L⁻¹. The fortified milks were gently mixed and stored for 72 h at 4 °C. Meanwhile, the stability of microcapsules, color and sensory characteristics of fortified milk were determined in 24 h intervals.

RESULTS AND DISCUSSION

Encapsulation efficiency As it can be seen in Table 1, the encapsulation efficiency increased with decreasing Tween 80 and core:coat weight ratio in which the highest microencapsulation efficiency (85%) was achieved at an iron to lipid weight ratio and Tween 80 concentration of 0.04 and 5%, respectively. With regard to Tween 80, it is noteworthy that according to the Lichtenberg's model with increasing its concentration the liposomes can partially or entirely dissolves and this process leads to partial or complete damage of liposomes and finally leakage of their contents (entrapped irons).

Table 1: Influence of some variables on the efficiency of liposome method ($p < 0.05$).

Microencapsulation efficiency (%)		Iron:lipid ratio (w/w)	Tween 80 in wall (%)
Ferric ammonium sulfate	Ferrous sulfate		
66 ^e	69.2 ^e	0.10	10
74.2 ^d	77.3 ^{cd}	0.04	10
77.1 ^{cd}	80.4 ^{bc}	0.10	5
82.1 ^{ab}	85.5 ^a	0.04	5

Figure 1 also shows the effect of PGMS: iron salt ratios over a wide range on the efficiency of microencapsulation in F.A.E. technique in which with increasing the ra-

tio up to 15:1 (w/w) the efficiency improved whereas at any higher levels it reduced again. Therefore, the maximum value of microencapsulation efficiency (81.8%) was seen at a coat:core ratio of 15:1.

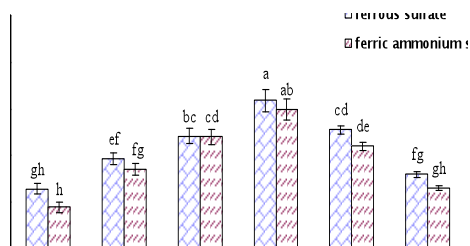


Fig. 1: Influence of coat:core ratio on microencapsulation efficiency of iron in F.A.E. method ($p < 0.05$).

In terms of the effect of encapsulation technique on the morphology of microcapsules, we attempted to take some photographs by electronic microscope, where due to the liquid form of samples it was almost impossible. As a result, we had to consent on the photographs were taken by light microscope (Fig. 2) which demonstrates an irregularly spherical shape with smooth surface for the microcapsules.

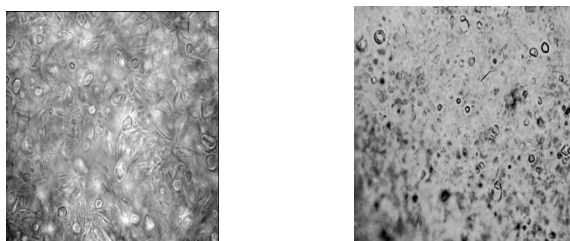


Fig. 2: Microscopic observations of ferrous sulfate microencapsulated by a) F.A.E. b) liposome (1000X)

Stability of microcapsules The ANOVA data showed that the considered parameters (storage period, iron concentration, method of iron preparation) had significant interaction (not shown). In the first hour of storage, TBA absorbance of milks fortified with microencapsulated irons (liposome, F.A.E., and commercial ones) did not increase with rising the iron ion concentration, while in the case of plain iron salt (non-encapsulated iron), the TBA absorbance significantly increased. Although, with rising the iron ion concentration (from 7 to 21 mg L⁻¹), the absorbance only increased 21% (from 0.0174 to 0.0213). But, interestingly during 3 days of storage at fridge conditions, the TBA absorbance of all samples increased ($p < 0.01$) on which it rose about 200 (0.0174 to 0.0514) to 320% (0.0213 to 0.0900) for plain iron salt, 85 to 135% for commercial, 87 to 140% for F.A.E., and 135 to 170% for liposome, respectively.

Effects of iron on the color and sensory properties The ANOVA data of L^* showed the interaction amongst vari-

ables (duration of storage, iron concentration, method of iron preparation, and type of iron salt) ($p < 0.01$). In all fortified milks, with increasing iron concentration the L^* significantly diminished, whereas during storage the L^* considerably increased. Meanwhile, the L^* of milks which were fortified with commercial and F.A.E. microencapsulated irons was significantly different in comparison with those fortified with plain and liposome microencapsulated irons. In addition, there was interaction between iron concentration and duration of storage, duration of storage and method of iron preparation, as well as type of iron salt and method of iron preparation on b^* parameter ($p < 0.01$).

The sensory evaluation data of milks fortified with different types of encapsulated and non-encapsulated iron during storage at 4 °C showed no significant difference in terms of astringency, bitterness, and color score whereas with regard to smell and metallic taste, considerable difference was found between samples and blank. In the first and fourth days of storage, milks fortified with non-encapsulated iron were significantly different with each other and blank from metallic taste point of view, while with regard to smell, at 7 mg⁻¹L and 14 mg⁻¹L, they did not have significant difference ($p > 0.95$).

CONCLUSIONS

The present study indicated that encapsulation efficiency of liposome and F.A.E. methods was dependent on Tween 80 concentration and iron: lipid weight ratio, respectively. TBA results also showed that the rate of lipid oxidation decreased significantly in milks fortified with microencapsulated irons regardless of microencapsulation methods. In addition, TBA absorbance showed a close dependency with the duration of storage and iron concentration. With regard to the sensory properties, the samples which fortified with F.A.E. microencapsulated iron and commercial liposome at 7 mg⁻¹L were fairly similar to control. The findings of color evaluation showed that L^* , b^* and a^* values are dependent on iron concentration, duration of storage, type of iron salt, as well as encapsulation methods. Therefore, according to the findings of the present study, the F.A.E. method was found as an applicable, feasible, rapid, efficient, and effective method for microencapsulation of various iron salts in order to be used in fortification of pasteurized milk with iron.

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