DEVELOPMENT OF A PROTEIN ENCASED FERROUS FUMARATE MICROCAPSULE

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ABSTRACT

Many iron fortificants have problems of imparting unacceptable colour and taste to the food product, decreasing the stability and bioavailability of iron. In this study, a novel method of developing a bioavailable iron fortificant is presented. Chelation and encapsulation are used to preserve iron in ferrous state, and also to minimize the unacceptable colour changes imparted by the fortificant in to food. Encapsulation was achieved using isoelectric precipitation of chickpea protein. The particles displayed encapsulation efficiency of 89.9 %, and loading capacity of 7.1 %. The percentage of Fe^{2+} encapsulated was determined to be 96.1 %. The microcapsules showed efficient release at pH 2, 4, 6 and 8. Over 75.0 % of iron was released at pH 2 within a 5 h period.

***Key words*:** microencapsulation, ferrous fumarate, chickpea protein

1. INTRODUCTION

Iron deficiency is one of the commonest nutrient deficiencies prevalent throughout the world. As a solution to this condition, fortification of staple food with iron containing compounds is carried out [1]. Since iron in ferrous state has better solubility than the ferric state, ferrous is proven to have better absorption than the ferric form [2]. However, iron in ferrous state is susceptible to oxidation by many factors including temperature, oxygen in air and alkaline pH. Therefore protection of the ferrous compound is necessary for the stability of the food product [3]. While both chelation and encapsulation have been reported to preserve ferrous in its oxidation state, encapsulation has the additional advantage of reducing the interactions between iron and food that may lower bioavailability of iron.

2. METHODOLOGY

2.1. Isolation of Chickpea Protein

Chickpea seeds were ground to a powder and defatted in hexane using a Soxhelt apparatus. Chickpea protein concentrate was obtained by alkaline solubilization at pH 12 followed by isoelectric precipitation at pH 4.5 [4].

2.2. Synthesis of Ferrous Fumarate

First, a solution of $FeSO_4$ was prepared by nitrogen purging 60 ml of water at 90 ^{0}C and dissolving 14.30 g of $FeSO_4.7H_2O$. Next a suspension of ferrous hydroxide was prepared by adding 4.25 g of 97 % NaOH dissolved in 10 ml of deionized water to the $FeSO_4$ solution over a period of 20 minutes while stirring. The ferrous hydroxide suspension was made air free by nitrogen purging again. Then, 6.38 g of fumaric acid was added in small portions to the ferrous hydroxide suspension while stirring at 100 ^{0}C. The precipitate formed was filtered off while hot, washed with 20 ml of water at 50 ^{0}C and dried overnight at 105 ^{0}C [5].

2.3. Encapsulation of Ferrous Fumarate

First, chickpea protein isolate (1.80 g) was dissolved in 50 ml of NaOH (pH 12) solution by stirring for 30 minutes. Then, 0.45 g of ferrous fumarate was added to the protein solution and stirred to obtain a suspension. After that, 0.1 M NaOH was added drop-wise to the solution while stirringand the pH was brought to 4.5 (isoelectric point of chickpea protein). The precipitate obtained was washed with an HCl solution at pH 4.5 and freeze dried. The isolate was characterized using FTIR spectrometer, particle size analyzer, X-ray diffractometer and polarized light microscope.

2.4. Determination of Optimal Core: Shell Ratio

Microcapsules with core: shell ratio 1:1, 1:2, 1:4, 1:6 and ferrous sulphate: ascorbic acid: protein ratio 1:1:5 (x) were obtained and encapsulation efficiency and percentage of ferrous in each sample was determined [3]. To determine the ferrous percentage a known amount of microcapsules was dissolved in pH 2 solution and reacted with NH_4SCN solution and absorbance was measured at 490 nm using a colorimeter. Then, another portion of the solution was oxidized using a $KMnO_4$ solution and the total amount of iron present was determined. Using the results, optimal core: shell ratio was selected for further analysis.

2.5. Determination of Fe^{2+} in Capsules

First, 0.04 g of microcapsules was dissolved in 20 ml of 1 M H_2SO_4 solution. Next the solution obtained was divided in to two solutions of equal volume. Then, one portion of the solution was oxidized by 0.1 M $KMnO_4$ solution added drop wise using a syringe. After that, 10 ml of 0.1 M NH_4SCN solution was added to each solution and absorbance readings for the $Fe(SCN)_3$ complex were obtained at wavelength 490 nm using a colorimeter. A calibration curve for $Fe(SCN)_3$ was obtained using $FeCl_3$ solutions of concentration 20, 40, 60, 80 and 100 in mg/l. Using the calibration curve and the absorbance readings, amount of total iron and Fe^{3+} present, and the Fe^{2+} percentage was determined [6].

2.6. Determination of Encapsulation Efficiency

First, 0.04 g of microcapsules was dissolved in 100 ml of 1 M HCl solution. Then, absorbance of the solution was determined using Atomic Absorption Spectrometer (AAS). A calibration curve for iron was constructed using $FeCl_3$ solutions of concentration 2, 4, 6, 8, 10 and 12 mg/l. Using the calibration curve, the amount of iron entrapped in the capsules was determined. Encapsulation efficiency (EE) was calculated using the following formula (01).

$$EE\ \% = \frac{\text{Entrapped amount of iron}}{\text{Amount of iron added}} \times 100\ \% \qquad (01)$$

2.7. Determination of Loading Capacity

First, 0.04 g of microcapsules was dissolved in 100 ml of 1 M HCl solution. Then, absorbance of the solution was determined using AAS. Loading capacity (LC) was calculated using the following formula (02).

$$LC\ \% = \frac{\text{Entrapped amount of iron}}{\text{Weight of microcapsules}} \times 100\ \% \qquad (02)$$

2.8. Release Study

First microcapsules were suspended in 5 ml of pH 2 citrate buffer solution inside a dialysis bag at 37 ^{0}C. Next, the dialysis bag was placed in a beaker containing 95 ml of pH 2 citrate buffer solution. Then, under continuous stirring 3 ml aliquots were drawn from the beaker at 30 min intervals and 3 ml of fresh solvent was added to replace the aliquots drawn. Finally, the amount of iron in each aliquot was determined using AAS. This procedure was repeated for the pH 4, 6, and 8 buffer media withthree different samples of microcapsules for 48 h.

2 RESULTS

3.1. Encapsulation Efficiency

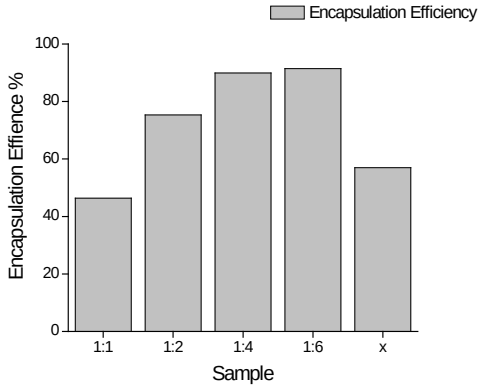

Figure 1: Encapsulation efficiencies of the microcapsule samples

The microcapsule samples with 1:4 and 1:6 core: shell ratios gave encapsulation efficiencies of 89.9 % and 91.4 %, as shown in figure 01.

3.2. Percentage of Fe^{2+}

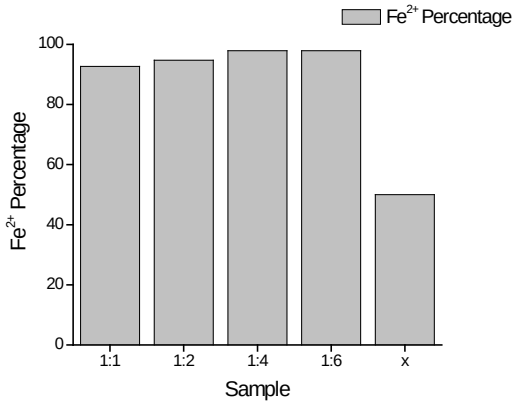

Figure 2: Fe^{2+} percentages of the samples

Percentage of Fe^{2+} was determined to be 97.9 % in the sample with 1:4 core: shell ratio and 97.9 % in sample with 1:6 core: shell ratio as shown in figure 02. Therefore as the optimal core: shell ratio 1:4 was selected for further testing.

3.3. Loading Capacity

Microcapsules showed average loading capacity of 7.1 %. Protein molecules have a closely knit network structure. This can be the reason for higher loading capacity in protein capsules.

3.4. Particle Size Analysis

The microcapsules show a range of particle sizes between 0.1 - 10 µm. This may be due to the presence of protein molecules with various chain lengths in the chickpea protein isolate.

3.5. Plane Polarized Light Microscopic Images

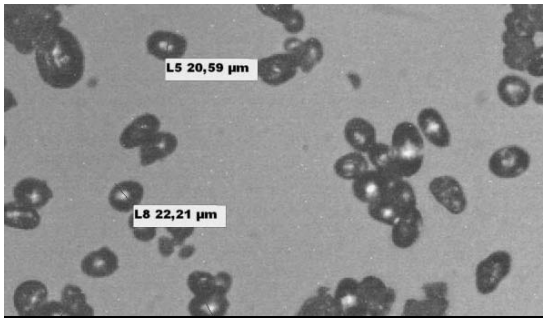

Figure 3: Plane polarized light microscopic image of microcapsules

The microcapsules were observed using the polarized light microscope under ×100 magnification. The image shown in figure 04 suggests that there is some extent of agglomeration of the particles.

3.6. Fourier Transform Infrared Spectra

In figure 04, the peak at 1254 cm^{-1} in the spectrum of ferrous fumarate (b) occurring due to the conjugated ester group is masked in the spectrum of microcapsules. The FTIR transmission spectra of protein and microencapsulated ferrous fumarate show similar peaks. Since the peak positions of microcapsule do not show significant influence by the core, the interactions between the core and the shell may not be strong.

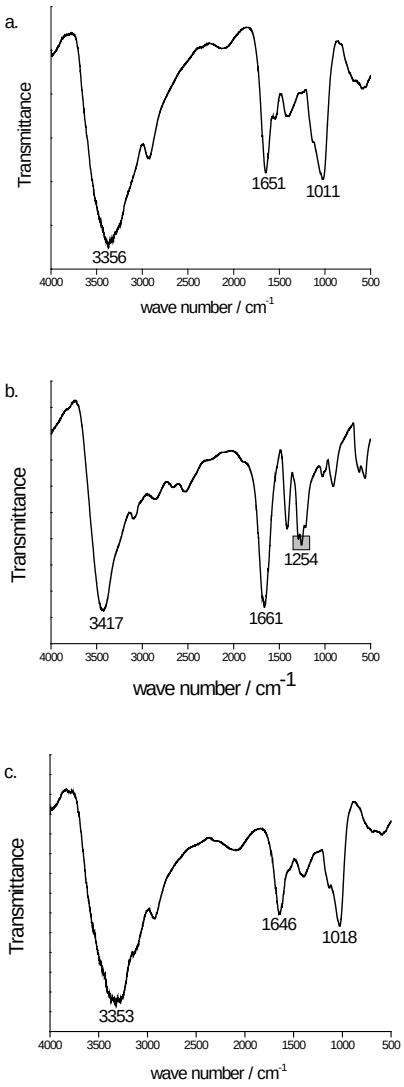

Figure 4: FTIR (a) chickpea protein (b) ferrous fumarate (c) Microencapsulate

3.7. Release Study

The highest release of iron from the protein shell is seen at pH 2 according to figure 05. At pH values 6 and 8, high and similar release is observed. Release at pH 4 is comparatively, low. The decrease in the efficiency of release at pH 4 may be attributed to the close proximity of the pH value to the isoelectric point of chickpea protein.

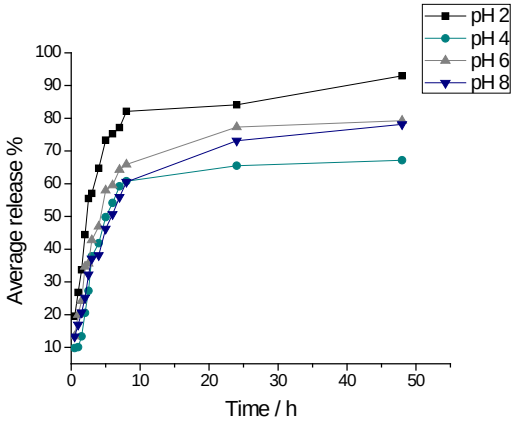

Figure 5: Average percentage release at four different pH values

4. CONCLUSIONS

The shell: core ratio of 4:1 showed encapsulation efficiency of 89.2 % and loading capacity 7.1 %. Also, the percentage of ferrous encapsulated was determined to be 97.9 %. Plane polarized light microscopic images indicated some agglomeration of the particles. This study yielded particles with hydrodynamic diameters in 0.1 -10 µm range. Using the spectra from X- ray diffractometer and FTIR spectrometer, the absence of strong chemical bonding between the core and the shell were established. Therefore, the drug can be released from the shell efficiently. The microcapsules showed >80% release at pH 2, 6, 8 after 48 h. At pH 4, ~ 60 % release was seen. At the close proximity of the isoelectric point of chickpea protein, less release was observed.

5. REFERENCES

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