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Extended Abstract

The Effect of Encapsulated Camel milk's Lactoferrin with Whey Protein Isolate-pectin Complexes on the Growth and Viability of MG63 Osteoblast Cell

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Abstract

Lactoferrin is one of the most important bioactive compounds that can increase the activity of the immune system and osteogenicity. However, it is sensitive to environmental stresses. Hence, the production of functional beverage including encapsulated lactoferrin by noncomplex of WPI/HMP was investigated in the current work. Nanocomplex was prepared by a complexation of whey protein isolate (WPI)-high methoxyl pectin (HMP) at total biopolymer concentration of 0.4% (w/w) at pH values of 3, 3.5 and 4 through post and pre-blending acidification procedures. In order to encapsulate lactoferrin, it was inserted to the WPI/HMP complex in which formed by the ratio 2:1 and pre-acidification (Pr-A).The results of Zeta-potential and particle sizes showed that by increasing HMP, the negative sites of the complex increased as well as the size of nano-particles. The effect of entrapped lactoferrin in WPI/HMP nano-particles on osteoblastic proliferation by MTT assay showed WPI/HMP+LF50 μg/mL a growth equal to the rate of positive control. For example the pure lactoferrin concentration of 50 μg/mL progressed 16% growth compared to the positive control. Therefore, it can be concluded that almost all lactoferrin in WPI/HMP+LF50 μg/mL combined or complexed and the cell access failed. The lactoferrin entrapped by WPI/HMP+LF100 μg/mL had a 113% growth and statistically occured at level of pure lactoferrin 50 μg/mL.

Keywords: Beverage, Lactoferrin, Osteoblast, Pectin, Whey Protein Isolate

Introductıon

Lactoferrin (LF) is a critical bioactive component which has a major contribution in the healthy foods, baby formula, and pharmaceutical industry. LF is a non-heme iron binding glycoprotein that belongs to the transferrin family. It has a molecular weight of 80 kDa containing an iron atom in its interior and amino acid residues along its surface, such as

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histidine and tryptophan. The encapsulation of bioactive compounds through nano-particles produced by association of two different biopolymers (e.g., protein-polysaccharide complex) can provide improved functional properties (Esfanjani *et al*., 2017; Garnero and Delmas, 2002; Hill *et al*., 1997). Application of the appropriate wall materials is a critical step in producing nano-particles. The main biopolymers which can be used for production of complexed nano-particle carriers are proteins such as β-lactoglobulin, zein, gelatin, soy protein, collagen, and albumin, and polysaccharides including pectin, alginate and chitosan (Hou *et al*., 2014; 2015). Whey protein is a natural emulsifier and stabilizer with the ability to produce thermodynamically and kinetically stable systems and its association with polysaccharides create a complex with thickening and steric stabilizing behaviors (Jafari *et al*., 2008). For example, pectin is one of the most popular polysaccharides which has been widely used in a complexed form with whey proteins for producing multilayer encapsulation systems (Jeney, 2017; Katouzian and Jafari, 2016; Kanwar *et al*., 2015). Pectin has been applied as a gelling, thickening and stabilizing agent in the food and pharmaceutical industries for many years. It is extracted from plant cell walls, especially from apple pomace, citrus fruits, and sugar beet root.

Therefore, the nano-particle complex of whey protein/pectin can be a good candidate for encapsulation of bioactive compounds. Our main goal of this study was to develop an optimized nano-particle formulation by whey protein-pectin complexes for entrapment and protection of LF. For this purpose, the main factors which could affect the properties of nano-particle were investigated including the ratios of whey protein-pectin, pH value, and preparation method in two forms of pH decrease before (pre-blending) and after (post-blending) mixing the biopolymer solutions.

Material and methods

Raw camel milk was provided from dromedary camels in Torkaman Sahra and was refrigerated until further experiments. Camel lactoferrin was extracted and purified according to our previous work (Naot *et al*., 2012). Whey protein isolate and high methoxyl pectin were purchased from Davisco and Merck, respectively. Other chemicals, reagents and solvents were all analytical grade and purchased from Merck.

Biopolymer solution preparation

The 2% (w/v) solutions of whey protein isolate (WPI) and high methoxyl pectin (HMP) were prepared, separately. According to the literature, a total biopolymer concentration of 0.4% (w/w) in acidic pH values (3, 3.5 and 4) was used (Mirhosseini *et al*., 2008). For complexion by post blending acidification, WPI and HMP stock solutions were initially diluted to concentrations corresponding to WPI: HMP ratios of 2:1, 1:1, and 1:2 for a total concentration of 0.4% (w/w). The diluted WPI and HMP solutions were adjusted to pH=7.0, mixed at the predetermined ratios and the pH of the final mixture (pH=7.0) was adjusted gradually to 4.0, 3.5, and 3.0 by addition of HCl (0.5 N) with gentle magnetic stirring for 3 min at each pH before decreasing the pH to the next value. Lactoferrin entrapment was done by combining (0.4% w/w) lactoferrin into HMP solution followed by adding WPI solutions at determined ratios; for instance in a 2:1 ratio of WPI:HMP, the final suspension was containing 0.27% WPI, 0.13% HMP and 0.1% lactoferrin.

The zeta potential and particle size at the liquid shear plane of the complexes was measured using a Zetasizer 2000. After 3 min stirring of WPI–HMP mixtures at a specific pH, 30 g samples were centrifuged at 8000 g for 30 min and the pellets were freeze-dried. The sedimentable-complex yield, or precipitation rate of the biopolymers in complex formation, was calculated. Complex-entrapped LF was determined by HPLC (Mohammadi *et al*., 2016). A freeze-dried pellet was suspended in 0.6 M TCA solution in order to reduce the pH value to

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1.0 and release LF as WPI-HMP complexes dissociate at this pH value (the positive charge of WPI molecules and the neutral charge of HMP molecules prevents their binding to LF molecules). LF was detected by a UV-visible detector at 280 and 320 nm and compared to a calibration curve for pure LF. The LF loading of the complexes was calculated.

Morphology, FTIR and DSC studies

Selected samples were chosen for microstructural analysis using scanning electron microscopy (SEM) and atomic force microscopy (AFM). The microstructure analysis of the samples was examined by Fourier transform infrared spectroscopy (FT-IR). The IR spectra of the samples were recorded by a FT-IR spectrophotometer (Avatar, Thermo Nicolet, USA) using the attenuated total reflection technique. The spectrum was scanned in transmission mode from 400 to 4000 cm^{-1} wavenumber range. Thermal behavior of WPI-HMP nano-particles was investigated by a differential scanning calorimete. Samples were scanned at the temperature range of 0–400 °C with the rate of 30 ^{o}C/min.

Statistical analysis

The experiments were all carried out in triplicate. The collected data were analyzed by randomized factorial design (ANOVA); the means were compared by Duncan's multiple range tests at the 95% level through SPSS version 21 (IBM, USA).

Results and discussion

The ζ-potential and z-average of complex nano-particles is shown in Figure (1). Our results revealed a decreasing ζ potential from pH=4 to 3 in the nano-particles produced by both methods of pre- and post-acidification. In the nano-complexed formed via pre-acidification method, the lowest ζ-potential was related to WPI/HMP formulation with a ratio of 1:2 at pH=3. For nano-particles produced by the post-acidification method, the ratios of WPI-HMP did not have a significant influence (P<0.05) on the ζ-potential of formed complexes at pH=4; but, in pH values of of 3.5 and 3, a significant effect (P>0.05) was observed. The lowest ζ-potential in all treatments was found for the nano-particles produced by WPI-HMP with the ratio of 2:1 at pH=3 (Figure 1).

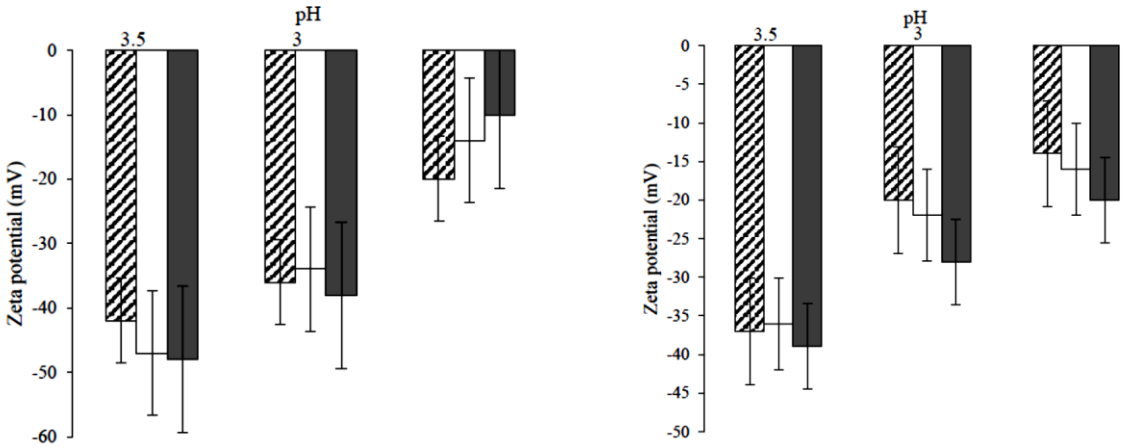

Figure 1. Zeta potential (mV) of WPI–HMP nano-complexes at the ratios of 2:1, 1:1 and 1:2: (left) pre-blending acidification and (right) post-blending acidification

In both post- and pre-acidification methods, the sedimentable-complex yield was increased at higher ratios of WPI and decreased by increasing pH value from 3 to 4 (Figure 2). This can be explained by better interaction of negative surface charges of pectin and positive surface charge of whey proteins when decreasing the pH value (Raei *et al.*, 2015). Also, as shown in

Figure (2), the sedimentable-complex yield in the prepared complex nano-particles by pre-acidification method was higher than post-acidification.

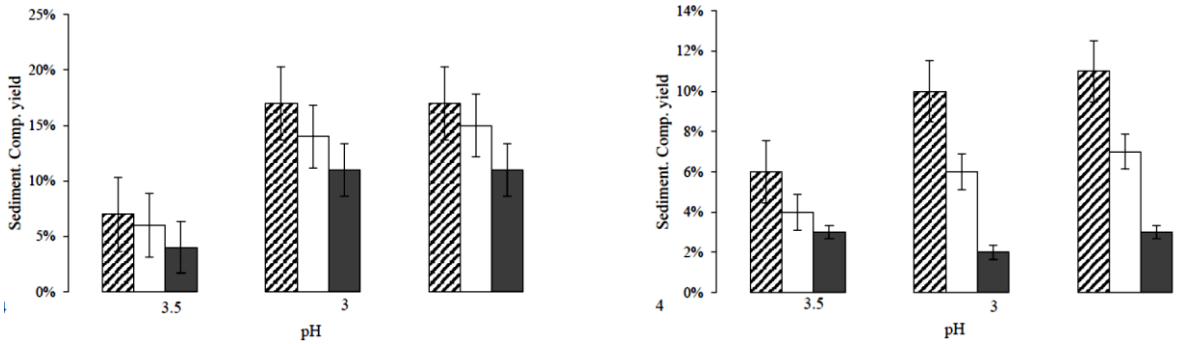

Figure 2. Sedimentable complex yield (%) of WPI–HMP nano-complexes at ratios of 2:1, 1:1 and 1:2: (L) pre-blending acidification and (R) post-blending acidification

Conclusion

The results of this study showed that LF can be loaded in the biopolymer complexes of whey protein isolate and high methoxyl pectin at pH values of 3 to 4. This interaction was influenced by pH, the ratio of WPI-HMP, and the preparation method (including post- and pre-acidification). The smallest particle size was provided by applying WPI-HMP in a ratio of 2:1 through post-acidification at pH= 3.5. Production of large particles by post acidification could be related to the gradual acidification which allows more time for complexes to rearrange in a compact structure. The sedimentable-complex yield of nano-particles was increased at lower pH values due to better interaction of negative HMP molecules with the positive WPI surface. Also, the pre-acidification method provided higher sedimentable-complex yield, more LF loading, and higher encapsulation efficiency. In general, our results introduced the nano-particles prepared by WPI-HMP complex at a ratio of 2:1 through pre-acidification method in pH=3.5 as an optimal nanocarrier for LF which can be used in the food formulations and pharmaceutical industries.

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