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Gelatin as texture modifier and porogen in egg white hydrogel

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ABSTRACT

In the present study, composite egg white/gelatin hydrogels were produced and their porosity was increased through the subsequent removal of gelatin by leaching into water. The composite gel with 0.5% gelatin showed a higher degree of swelling than did the control gelatin-free sample after 60 min of immersion in an aqueous medium which was ascribed to the formation of capillary channels due to gelatin leaching. The composite gel containing 0.3% gelatin showed the highest water-holding capacity and firmness indices among all samples. Gel porosity decreased with increasing gelatin content. However, after gelatin depletion, higher concentrations of gelatin yielded hydrogels with higher porosity, as confirmed by scanning electron microscopy. Based on Fourier transform infra-red spectroscopy, it was concluded that the count of hydrogen bonds decreased after gelatin depletion. X-ray diffraction analysis indicated that intermolecular interaction between gelatin and egg white proteins had taken place in the amorphous phase.

1. Introduction

Egg white proteins are extensively used in food products, especially in edible gels, owing to their high nutritional value and versatile techno-functional properties (Nyemb et al., 2016). Egg white encompasses a variety of globular proteins, such as ovalbumin (54%), ovotransferrin (12%) and lysozyme (3.5%), contributing to its heat-induced gelation property. Thermal treatment above denaturation temperature exposes non-polar and sulfhydryl-containing amino acids of egg white proteins, which allows for hydrophobic and disulfide interactions. The formation of such interactions and bonds leads to the formation of a strong viscoelastic hydrogel (Gu et al., 2017). The gelling ability of egg white was employed to modify the consistency and textural properties of food systems, as well as to design matrices for delivery of bioactive ingredients (Su et al., 2015), such as α-tocopherol, an important antioxidant (Somchue, Sermsri, Shiowatana, & Siripinyanond, 2009). However, the weak mechanical strength of egg white gel limits its application in fabrication of advanced materials (Nojima & Iyoda, 2018). Mixing of egg white proteins with other biopolymers, to produce mixed or composite gels, can be helpful to resolve the issue. Blending of egg white with different biopolymers, such as hydroxypropylmethylcellulose (van den Berg, Jara, & Pilosof, 2015), konjac glucomannan (Hu et al., 2016; Liu et al., 2013), and soy protein isolate (Su et al., 2015), has been proposed as a potential approach to produce mixed gels with modified physicochemical, mechanical, and textural properties.

Hydrogels are one of the most promising encapsulation systems. They are three-dimensional polymeric networks and have high ability to retain water or biological fluids (Somchue et al., 2009). The size and architecture of pores can affect the properties of hydrogels, especially in the case of tissue engineering and drug delivery (Annabi et al., 2010). Several advantages, such as enhanced surface area, a more open structure, higher and faster swelling, as well as rapid response to external stimuli, were reported for the hydrogels with larger pore sizes, known as porous gels, over their conventional counterparts with low porosity (Guo et al., 2013). Different methods have been used to produce porous structures, such as thermally-induced phase separation, solvent casting, gas foaming, freeze-drying, electrospinning and porogen (porosity generator) leaching (Tang et al., 2012). Porous hydrogels can be formed via the addition of porogen agents during gelation and their subsequent depletion. Solid (such as silica particles, ice crystal, sodium chloride and gelatin), liquid (such as organic solvents) and gas (such as inert gas) templates were used as porogens to produce hydrogels and structures with enhanced porosity (Guo et al., 2013).

Gelatin is a natural, biodegradable animal protein produced from collagen by acid- or alkali-catalyzed hydrolysis and is widely used for a variety of biological and functional purposes in cosmetic, pharmaceutical and food formulations (Sahoo, Sahoo, Biswas, Guha, & Kuotsu, 2015). Hydrogels made of gelatin are thermally reversible, due to the important role of intermolecular hydrogen bonds in the gelation of

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gelatin. These gels melt around human body temperature, which gives them a unique characteristic, well-known as melt-in-mouth property (Dille, Hattrem, & Draget, 2018). Gelation of a gelatin solution, and subsequent changes in the network of hydrogel, results from the partial return of disordered gelatin molecules (coil) to the collagen-like structure (Pang, Deeth, Sopade, Sharma, & Bansal, 2014). Addition of gelatin to edible protein gels was suggested as a way to improve their techno-functional properties, such as water holding capacity (WHC). In this regard, the presence of gelatin at concentrations ≥1% prevented the expulsion of serum from acid milk protein gels, which could be valuable in the manufacture of food products such as yoghurt (Pang, Deeth, Sharma, & Bansal, 2015). Moreover, owing to its temperature-dependent gelation and solubilization property, gelatin was employed as a porogen agent to produce porous structures such as highly porous poly(lactic-co-glycolic acid) (PLGA) microparticles (Kim, Park, & Kim, 2015) and scaffolds (Tang et al., 2012).

In spite of its proven performance as a porogen (Cuadros, Erices, & Aguilera, 2015) and texture-modifier (Brink, Langton, Stading, & Hermansson, 2007), there has been no report in the literature, about the application of gelatin in fabrication of binary egg white protein/gelatin gels. Moreover, it has not been employed as a porogen to make porous egg white hydrogel. Accordingly, in the present study, mixed gels, composed of egg white proteins and different concentrations of gelatin, were produced in a two-stage procedure. The procedure included the heat coagulation of egg white proteins, followed by cooling the resultant heat-set gels to trigger gelatin gelation. Subsequently, the gelatin was depleted via leaching into water at 40 °C to yield porous egg white hydrogel. Characteristics of mixed and gelatin-depleted hydrogels were determined.

2. Materials and methods

2.1. Materials

Whole eggs were purchased from a local store in Karaj, Iran. Egg whites were separated carefully from the egg yolks and chalaza. The protein content of egg white was determined by the Kjeldahl method (N × 6.25, 10.5%). Separated egg whites were stored at −18 °C until used. Type B gelatin with Bloom strength of 230–250 was obtained from Pariznova Company (Tehran, Iran). The enzyme pepsin (activity of ≥3000 units mg^{-1}) was obtained from Bio Basic (Bio Basic Inc., Canada). Hydrochloric acid and sodium chloride were purchased from Merck (Darmstadt, Germany). Ethanol was obtained from Zakaria Jahrom Company (Tehran, Iran). All solutions were prepared in distilled water.

2.2. Preparation of heat-set egg white/gelatin hydrogels

For the preparation of hydrogels, the pH of egg white solution with a protein concentration of 10% was adjusted to 7.0 with 2.0 M HCl. Subsequently, this solution was charged with different concentrations (0, 0.3, 0.4 and 0.5%) of gelatin powder and stirred for 20 min under mild conditions. The binary solutions were heated for 30 min at 80 °C to form heat-set gels (Nyemb et al., 2016). Immediately after heating, the samples were cooled with ice water and stored at 4 °C for 18 h before analysis.

For gelatin leaching, the mixed egg white protein/gelatin hydrogel samples (≈3.5 g) were immersed in 20 ml of distilled water at 40 °C for 2.5 h. The gelatin-depleted samples were also analyzed for porosity, microstructure, X-ray diffraction (XRD) and chemical interactions.

2.3. Swelling study

For assessing the swelling property, mixed egg white/gelatin hydrogel samples (≈2.0 g) were placed in 15 ml of enzyme-free simulated gastric fluid (SGF), consisting of 0.2% sodium chloride and 0.7% hydrochloric acid (37%) with the final pH of 1.2 for up to 150 min at 37 °C (Maltais, Remondetto, & Subirade, 2010). Periodically, specimens were removed from the solution, blotted dry and accurately re-weighed. Eq. (1) was used to determine the percentage of swelling:

$$\text{Swelling}(\%) = \frac{W_t - W_0}{W_0} \times 100 \quad (1)$$

where W_t and W_0 are, respectively, the weight of swollen gel at time t and initial weight of gel samples.

2.4. In vitro gastric degradation

The *in vitro* gastric degradation of hydrogel samples was studied according to the method of Maltais et al. (2010) with some adjustments. For the degradation experiment, about 2.0 g of gel samples were incubated in SGF with pepsin (3.2 g l^{-1}) at 37 °C under continuous shaking at 100 rpm. The final pH of SGF (consisting of sodium chloride, hydrochloric acid, and distilled water) was 1.2. The concentration of released protein in the simulated gastric medium during 2.5 h was determined by measuring the protein content of centrifugal (1500 × *g* for 10 min) supernatants, using the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). Finally the extent of gel degradation was calculated, using the following equation:

$$\text{Matrixdegraded}(\%) = \frac{\text{releasedprotein}}{\text{Totalproteinconcentrationinthegel}} \quad (2)$$

2.5. Textural analysis

For textural analysis, at first the mixed gel samples were equilibrated at room temperature (25 ± 2 °C) for 2 h prior to characterizations. After that, the firmness of mixed egg white protein/gelatin hydrogels was determined by a penetration test, using a universal texture analyzer apparatus (M350-10CT, Testometric, Lancashire, UK). Gel samples with 13 mm diameter and 30 mm height were penetrated by a 7.0 mm-diameter cylindrical stainless steel probe to a depth of 20 mm at a speed of 60 mm min^{-1}. Firmness is expressed as the maximum force (N) required for penetrating the samples.

The compressive stress of the mixed hydrogels, as a measure of their strength, was assessed by the uniaxial compression test. For this purpose, gel samples were cut into 2 cm × 2 cm cylinders and compressed to 75% of their initial height, using a flat plate plunger at a constant crosshead speed of 1 mm s^{-1}. Compressive stress (σ_c) was calculated according to the following equation:

$$\sigma_c = \frac{F}{A} \quad (3)$$

where F is the force at fracture point (N) and A is the cross-sectional area of gel samples (m^2).

2.6. Water-holding capacity and syneresis

WHC of egg white heat-set gel samples containing different concentrations of gelatin (0, 0.3, 0.4 and 0.5%) after 24 h of storage was determined through a centrifugation method. For this purpose, the gel samples, which are fabricated within 5.0 ml tubes, were centrifuged (Universal 320, Hettich Zentrifugen, Germany) for 10 min at 1500 × *g* and WHC was calculated using the following equation (4):

$$\text{WHC}(\%) = 100 \times \left[1 - \left(\frac{\text{seperatedwaterweight(g)}}{\text{waterweightinthegel(g)}}\right)\right] \quad (4)$$

For the measurement of syneresis, the fresh mixed gel samples were stored upside down for 2 h at room temperature (24 ± 2 °C) and the expelled water from the samples was quantified.