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Gelling and bile acid binding properties of gelatin-alginate gels with interpenetrating polymer networks by double cross-linking

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ABSTRACT

Interpenetrating polymer network (IPN) is an effective method to improve functional properties of hydrogels by forming cross-linking networks. In this study, the gelatin-alginate gels formed by the combination of enzymatic and ionic cross-linking were called IPN gels. Meanwhile, the gels with the treatment of only transglutaminase (TG) or Ca^{2+} were named as G-semi-IPN and A-semi-IPN, respectively. The formation of semi-IPN and IPN was confirmed by studies on rheology, thermodynamics and micro-morphology. The results showed that the IPN gels had improved gelling properties and structural stability. The functional properties of different gelatin-alginate gels were also investigated. It was firstly found that the IPN gels could enhance mechanical properties, decrease swelling capacity and had better bile acid binding capacity. These results of gelatin-alginate gels provide references and novel prospects of IPN for the application in the field of food industry.

1. Introduction

Food gels have been applied to the following aspects: new food products, food additives, edible packaging films and delivery systems (Dalodom et al., 2016; Deng, Kang, Liu, Feng, & Zhang, 2017; Jridi et al., 2014; Nedeljković et al., 2017). Recent studies have focused on the preparation of food gels using natural polymers such as gelatin, alginate and chitosan, due to their wide resources, low cost, biodegradability and biocompatibility (de Britto et al., 2014; Wen, Lu, & Li, 2014c). However, most food gels are a simple composite of several substances. It has been proved that cross-linking is an effective means to improve the functional properties of hydrogels. Aldehyde cross-linking agents (e.g. glutaraldehyde) are of cytotoxicity, so natural cross-linking agents (e.g. transglutaminase) are more popular nowadays (Wen, Lu, & Li, 2014b). Transglutaminase (TG) can catalyze the formation of amide bonds between glutamine and lysine residues on the protein and enhance the space structure of gelatin, resulting in improvements in water resistance, thermal, and morphological properties (Hong & Xiong, 2012; Liu et al., 2017). Calcium ions can form an "egg-box" structure with α-L-guluronate (G block) of alginate, thus forming a nontoxic cross-linked gel (Maestrelli, Zerrouk, Cirri, & Mura, 2015).

With the continuous development of the application of food gels, IPN technology has gained interest from researchers in order to meet high requirements for gel properties. Interpenetrating polymer network, namely IPN, is to form intertwined networks by two or more polymers. If only one polymer was cross-linked and the other was still the sol state, the formed gel is a semi-IPN (Wen et al., 2014c). It has been reported that semi-IPN or IPN gels have enhanced salt resistance and gel strength (Wang et al., 2016; Wen et al., 2014c).

In the present research, gelatin-alginate gels with semi-IPN or IPN structure were prepared by cross-linking gelatin with TG or/and cross-linking alginate using Ca^{2+} aiming to achieve some improved functional properties. Studies on rheology, thermodynamics and micro-morphology were performed to demonstrate the formation of semi-interpenetrating and interpenetrating networks. The mechanical properties, hydration properties and bile acid binding capacities of gelatin-alginate gels were measured to evaluate their beneficial functions and to provide novel references for applications in food industry.

2. Materials and methods

2.1. Materials

Gelatin (from porcine skin, type A) and alginic acid sodium salt (sodium alginate) were purchased from Sigma-Aldrich (St. Louis, USA). Transglutaminase (TG, 100 U/g) was kindly donated by Jiangsu Yiming Biological Co., Ltd. (Jiangsu, China). Calcium chloride was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China).

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Table 1
The composition of gelatin-alginate gels preparation.

No.	Sample	Gelatin (mg)	Alginate (mg)	Ratio	TG(mg)	H_2O (μL)	$CaCl_2$ in the gel (mM)
Gel 1–1	G-A complex	300	60	5:1	0	2000	–
Gel 1–2	A-semi-IPN	300	60	5:1	0	2000	31.3 ± 0.5
Gel 1–3	G-semi-IPN	300	60	5:1	200	2000	–
Gel 1–4	5:1 IPN	300	60	5:1	200	2000	16.5 ± 0.5
Gel 1–5	1:1 IPN	60	60	1:1	40	2000	34.1 ± 1.2
Gel 1–6	0.2:1 IPN	12	60	0.2:1	8	2000	47.4 ± 0.2

Other reagents used in the experiments were of analytical grade.

2.2. Rheological testing

2.2.1. Preparation of sample solutions

According to the formula of Table 1, the sample solutions of G-TG, G-semi-IPN and IPN were prepared by diluting the concentration 1.5 times for rheological testing. Briefly, 100 g of gelatin and 20 g of sodium alginate were dissolved with 1000 μL of distilled water and magnetic stirred in water bath at 55 °C for 15 min in order to obtain G-TG solution. Then G-semi-IPN solution was obtained by adding 67 mg of TG to G-TG solution and the obtained solution was with 6.7 U/mL of transglutaminase. IPN solution was prepared by dissolving the same gelatin and sodium alginate into 900 μL of distilled water and adding TG and 100 μL of $CaCl_2$ solution (50 mM).

2.2.2. Oscillatory dynamic testing

The sample solution was immediately transferred to the standard steel parallel plate (40 mm diameter) preheated to 40 °C. The time sweep of 40 min was conducted on an ARG2 rheometer (TA Instrument, USA). The testing parameters were set as follows: test temperature as 40 °C, strain of 1%, frequency of 1 Hz and gap of 500 μm.

2.3. Preparation of hydrogels

The certain amount of gelatin and sodium alginate in Table 1 were mixed into 2 mL of distilled water and incubated at 55 °C for 15 min. TG was then added into the solution (10 U/mL) and wrapped with aluminum foil followed by reacting in oven at 40 °C for 30 min. After cooling to room temperature, the gels were immerged with 50 mM $CaCl_2$ solution (2 mL) and then horizontally oscillated for 1 h. The final concentration of Ca^{2+} in each gel was different due to different tightness of the structure and the volumes, as shown in Table 1. The gels were stored in the refrigerator at 4 °C for 24 h for the later characterization.

2.4. Differential scanning calorimetry (DSC)

The freeze-dried gels were crushed into fine particles and dried for another 48 h in oven at 30 °C. About five mg of samples were weighed for testing on a DSC 204F1 (Netzsch, Germany). An empty Al_2O_3 crucible was set as a reference and nitrogen was used as an atmosphere at a flow rate of 20 mL/min. The first heating scan was from 20 to 150 °C at a rate of 10 °C/min to observe the melting of samples. After cooling at the same rate, the second heating scan was run at the rate of 10 °C/min from 20 to 250 °C to detect the isomerization of samples.

2.5. Scanning electron microscopy (SEM)

The gels were immersed in the distilled water for 24 h to reach the swelling equilibrium and then frozen immediately in liquid nitrogen before vacuum drying. The dried samples were fixed to the double-sided adhesive tape followed by sputter-coating with gold for 45 s in order to make them conductive. Each sample was observed at an acceleration voltage of 5.00 kV and a magnification of 2000 by Sirion 200 SEM (FEI Company, USA).

2.6. Mechanical testing

The gels in Table 1 were prepared and placed on a TA-XT plus texture analyzer (Stable Micro Systems, UK) for texture profile analysis test (TPA test). The P/0.5 probe was used and the strain was set as 50%. The pre-test, test and post-test speed were 2 mm/s, 0.5 mm/s and 3 mm/s, respectively.

2.7. Hydration properties

2.7.1. Water holding capacity (WHC)

According to the method of Yadav, Kale, Hicks, and Hanah (2017) with some modifications, 0.1 g particles of freeze-dried gels were put into a centrifuge tube containing 20 mL of water and stirred up for 24 h. After centrifugation, the supernatant liquid was removed. The water holding capacity (g/g sample) was weighed and calculated by the following formula:

$$\text{WHC} = (\text{wet weight - dry weight})/\text{dry weight} \quad (1)$$

2.7.2. Freeze-thaw stability

Freeze-thaw stability of a gel was investigated by syneresis rate during repeated freeze-thaw cycles according to the method described by Muadklay and Charoenrein (2008). About 1 g of wet gels were placed in centrifuge tubes and centrifuged at 1000*g* for 8 min in order to remove the supernatant and water on tube walls. The gels were weighed as the initial weight. Then the tubes were frozen at −20 °C for 24 h and thawed at 30 °C for 2 h. The tubes were centrifuged to remove water and weighed again. The freeze–thaw cycle was repeated for up to five times. Syneresis rate (%) of the sample was the ratio of the separated water to the initial gel weight.

2.7.3. Swelling capacity

To investigate swelling behaviors at different pH values, wet gels were placed in an aqueous solution at different pH (1.0, 3.0, 5.0, 7.0, and 9.0 respectively) for 24 h to achieve swelling equilibrium. The dry weight of samples was weighed after freeze-drying. The swelling capacity (g/g sample) was calculated according to the following formula:

$$\text{Swelling ratio} = (\text{swelling weight - dry weight})/\text{dry weight} \quad (2)$$

2.8. Bile acid binding capacity

According to the method used by Feng, Dou, Alaxi, Niu, and Yu (2017), the bile acid binding capacities of gels were measured. In brief, about 10 mg of the prepared gels and cholestyramine resin (as the positive control) were added to 0.01 M HCl solution to react in the simulated gastric condition (37 °C for 1 h) and then neutralized with NaOH solution. The chenodeoxycholic acid (CDCA) stock solution dissolved in 0.01 M phosphate buffer at pH 7 was added and incubated in the simulated intestinal environment (37 °C for 1 h). According to the color reaction of unbound CDCA with NAD, NBT, 3-α HSD and diaphorase (room temperature in dark for 1 h), the absorbance value was

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