Food Chemistry 272 (2019) 694–701

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The preparation and physiochemical characterization of rapeseed protein hydrolysate-chitosan composite films

Cheng Zhang[a], Zhigao Wang[b], Yang Li[a], Yijie Yang[a], Xingrong Ju[a,b], Rong He[a,*]

[a] College of Food Science and Engineering/Collaborative Innovation Center for Modern Grain Circulation and Safety/Key Laboratory of Grains and Oils Quality Control and Processing, Nanjing University of Finance and Economics, Nanjing 210023, China
[b] College of Food Science, Jiangnan University, Wuxi 214122, China

ARTICLE INFO

Keywords:
Rapeseed protein
Hydrolysis
Chitosan
Edible film
Microstructure

ABSTRACT

The composite films were prepared by mixing rapeseed protein hydrolysate with chitosan. Upon increasing the degree of hydrolysis, rapeseed protein enhanced its compatibility with chitosan, thus making composite films denser. The tensile strength of films was increased from 16.04 to 23.46 MPa with increasing the degree of hydrolysis from 0% to 12%. Moreover, addition of chitosan enhances the mechanical properties of the rapeseed protein films, the α-helix content in the secondary structure of the rapeseed protein from 15.4% to 25.0%. And it is hydrogen bonding, the main force between two components that contributed to good compatibility, which supported by analyses of Fourier transform infrared spectroscopy and scanning electron microscopy. The results of the antibacterial properties of the composite film with 12% degree of hydrolysis were better compared with the chitosan film. Taken together, our results provide insights for the further application of rapeseed protein in making edible films.

1. Introduction

In recent years, research on edible film is becoming more and more popular. Proteins (e.g., soy protein, wheat protein, and kidney bean protein), polysaccharides (starch, cellulose, and chitosan) and lipids are the main components of edible film (Han, Yu, & Wang, 2018; Hassan, Chatha, Hussain, Zia, & Akhtar, 2017; Sun et al., 2017). Compared with synthetic packaging materials in the chemical industry, these biopolymer-based films have the potential to replace petroleum-based plastic packaging films and reduce environmental impact (Khalil et al., 2017). In particular, protein-based edible films are more popular due to their nutritional values and have good food sensory characteristics. In general, the mechanical properties of protein edible films are poor but can be modified by physical, chemical or enzymatic methods to strengthen the performance of the protein film. On the other hand, edible films of polysaccharides are chemically stable and can be adapted for long storage times and storage under different environments with good physical and mechanical properties (Cao, Liu, & Wang, 2018). In addition, some studies have shown that edible films made from composites (proteins and polysaccharides together) have better mechanical properties and water vapor permeability than those made from one component (Yoo & Krochta, 2011). Therefore, considering the limitations of edible films made from a single material, the future direction is to develop composite films with new edible materials (Hassan, Chatha, Hussain, Zia, & Akhtar, 2017).

Using rapeseed protein isolate (RPI) to make edible films has several advantages. First, RPI consists of all essential amino acids with higher value of nutrition (Adewole, Rogiewicz, Dyck, & Slominski, 2016). Second, rapeseed meal produced by rapeseed oil is the main byproduct of rapeseed oil processing, which contains many proteins. Currently, rapeseed meals are largely unused after oil processing and the reuse of rapeseed meals is of great significance in terms of economic benefits (Pudel, Tressel, & Düring, 2015). Third, RPI has film-forming properties. Some studies have shown that RPI can be used for making edible films (Jang, Shin, & Song, 2011; Jang, Lim, & Song, 2011). However, the poor mechanical properties of rapeseed protein-made edible films have limited their application.

Chitosan (CH) is a natural macromolecular compound that is abundant in the shell of shrimp and crab. CH not only has good film-forming property, biocompatibility, biodegradability, and non-toxicity but also has good antimicrobial properties that can inhibit the growth of some pathogenic microorganisms (e.g., *Escherichia coli and Staphylococcus aureus*) (Sayari et al., 2016). Chitosan has been used to enhance the mechanical and antimicrobial properties of RPI film. However, chitosan is only soluble in an acidic medium and has good antimicrobial activity at a pH value of 6 or so (Huang & Peng, 2015). In

* Corresponding author.
E-mail address: rong.he@njue.edu.cn (R. He).

https://doi.org/10.1016/j.foodchem.2018.08.097
Received 3 June 2018; Received in revised form 21 August 2018; Accepted 21 August 2018
Available online 23 August 2018
0308-8146/

contrast, under acidic conditions the RPI easily precipitates, so it is necessary to improve solubility especially in the low pH and compatibility with CH.

In this study, the solubility of rapeseed protein in acidic condition was improved through the limited hydrolysis of RPI to obtain rapeseed protein hydrolysate (RPH). The effects of incorporating CH on the physical and antimicrobial properties of RPH films were evaluated. RPH-CH composite films were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), total color difference (△E), tensile strength (TS), elongation at break (EB), and water vapor permeability (WVP). Moreover, the effect of protein hydrolysis and the different ratios of RPH to CH on the optical properties, mechanical properties and water permeability of the composite films were examined. The content of second structure of the proteins in the film; the microscopic structure and the antimicrobial activity of the RPH-CH composite films were also investigated.

2. Materials and methods

2.1. Materials

Rapeseed was supplied by COFCO East Ocean Oil & Grains Industries Co., Ltd., (Zhangjiagang, China). Chitosan was purchased from Solarbio life sciences Co., Ltd., (Beijing, China), its degree of deacetylation was greater than 90% and the molecular weight 50,000–190,000 Da. *Escherichia coli* (CICC 10899), *Bacillus subtilis* (CICC 10732) and *Staphylococcus aureus* (CICC 10001) were purchased from China Center of Industrial Culture Collection (CICC). Unless stated otherwise, all chemicals were of analytical grade and were purchased from Sigma-Aldrich Co., Ltd., (Shanghai, China).

2.2. Preparation of rapeseed protein isolate (RPI)

RPI was extracted from de-oiled rapeseed protein meal (RPM) according to a previously reported method with some modifications (Wang, Ju, He, Yuan, & Aluko, 2015). De-oiled RPM was dissolved in deionized water (1:15, w/v) by using 1 M NaOH to adjust the pH to 11.0 with stirring for 2 h at 50 °C. The slurry was centrifuged at 10,000*g* for 20 min at 4 °C, and the supernatant was collected. The pH value was adjusted to 4.5 to precipitate the proteins at room temperature for 1 h and then centrifuged to pellet the proteins. The protein was washed with anhydrous ethyl alcohol to remove the polyphenolic components. The recovered protein pellets were dispersed in a suitable amount of deionized water and the pH was adjusted to pH 7.0 with 1 M NaOH. Finally, the protein solution was freeze-dried to acquire RPI.

2.3. Restricted hydrolysis of rapeseed protein isolate

To improve the solubility of protein in acidic pH, the RPI needed to be properly hydrolyzed and the degree of hydrolysis needed to be controlled (Chabanon, Chevalot, Framboisier, Chenu, & Marc, 2007). In this experiment, alkaline protease was used to hydrolyze the rapeseed protein isolate and the reaction conditions for alkaline proteases was at 50 °C under pH 8.0 (He, Girgih, Malomo, Ju, & Aluko, 2013). An amount of RPI was dissolved in deionized water to make a 5% protein solution. The protein solution was heated to 50 °C, and the pH value was adjusted to pH 8.0 prior to the addition of the alkaline protease (4% of the RPI). During the reaction, the pH value of the reaction mixture was kept constant by the addition of 1 M NaOH. The amount of NaOH added was recorded to calculate the degree of hydrolysis of the protein. The degree of hydrolysis of the protein was controlled by the amount of NaOH added. After the reaction, the hydrolysate was heated to 90 °C for 10 min to inactivate the protease. After rapidly cooling in an ice-water bath, the hydrolyzed protein solution was adjusted to pH = 7, and the slurry was centrifuged at 10,000*g* for 20 min. The supernatant was collected and freeze-dried, and the rapeseed protein isolate was obtained. The hydrolysis degree of the protein is 3%, 6%, 9% and 12% of the protein hydrolysate was obtained.

2.4. Determination of degree of hydrolysis

The degree of hydrolysis (DH) in the hydrolysis of protein was determined by the pH-stat method, which was calculated as follows:

$$DH\% = \frac{B \times N_b}{M_p} \times \frac{1}{\alpha} \times \frac{1}{h_{hot}} \quad (1)$$

where B is the NaOH volume (mL) added during the reaction to keep the pH constant, N_b is the concentration of NaOH (mol/L), M_p is the amount of proteins (g), α is the average dissociation degree of α-NH_2 in hydrolysis and h_{hot} is protein hydrolysis degree constant. Rapeseed protein $1/\alpha = 1.1$, $h_{hot} = 8.04$.

2.5. Preparation of RPH-CH films

The RPH solution (2% w/v) was prepared by dissolving lyophilized RPH in deionized water at room temperature with mechanical stirring for 1 h. The CH solution (2% w/v) was prepared by dissolving CH in 1% acetic acid solution at room temperature and mechanically stirring overnight to mix well. The film-forming solution was prepared by mixing equal volumes of RPH and CH solutions, CH and RPH are soluble solids (total soluble solids content is 2%), and proper glycerol was added (20% of the soluble solids) as plasticizer. The solution was performed with ultrasonic treatment for 10 min, ultrasonic power is 65w and with magnetically stirring at 60 °C for 2 h. Then, 25 mL of the mixed solution was poured into a Teflon pane (10 cm × 10 cm × 1 cm). The solution in the Teflon pane was dried at 25 °C under 55% RH to form films with uniform thickness.

2.6. Characterization of the properties of the films

2.6.1. Thickness measurement

An electronic digital Vernier caliper (0.01 mm accuracy) (Guilin Guanglu Measuring Instrument Co. Ltd., China) was used to measure the thickness of the film. A film with smooth surface and uniform thickness was selected and some symmetrical sections were measured and averaged. Its value can be used to calculate and analyze the mechanical properties and water permeability.

2.6.2. Optical properties

Color was measured in transmission model with KONICA MINOLTA CM-5 (Japan) color meter with the EasyMatch QC software. Results were expressed as L^*, a^*, b^*, respectively, where L^* value was a measure of lightness and varies from −180 (black) to +180 (white), a^* value varied from −180 (green) to +180 (red), and the b^* value varied from −180 (blue) to +180 (yellow). The total color difference (△E) was calculated as follows:

$$\Delta E = ((L^*-L)^2 + (a^*-a)^2 + (b^*-b)^2)^{1/2} \quad (2)$$

where L^*, a^* and b^* are color values of standard color plate (Han, et al., 2018).

2.6.3. Mechanical properties

The mechanical properties of films mainly include tensile strength (TS) and elongation at break (EB), and those were measured by a texture analyzer (TA.XT2i Texture Analyzer, Stable Micro Systems, Godalming, UK). The tested film was equilibrated at 25 °C under 55% RH for 2 days. After that, the film was cut into 80 mm × 10 mm strips and was tested using a double-clamp probe with an initial grip separation of 60 mm at a testing speed of 1 mm/s (Du et al., 2016). Each film was measured at least five times and then averaged. TS (MPa) and EB (%) were calculated as shown in Eqs. (3) and (4), respectively.