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EFFECTS OF CHITOSAN AND MICROFLUIDIZATION PRETREATMENT ON PHYSICOCHEMICAL AND MECHANICAL PROPERTIES OF VICILIN-RICH PROTEIN ISOLATE (*PHASEOLUS AUREUS*) FILMS

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The aim of this research was to evaluate the effect of chitosan and microfluidization pretreatment on mechanical and microstructural properties of mung bean protein-based component (vicilin-rich) films. The microfluidization pretreatment led to a slight increase in denaturation temperature (Td). The film forming solutions showed a trimodal distribution with size at around 10 μm and 100 μm, regardless of chitosan and microfluidization. The presence of chitosan led to a significant increase in films thickness (FT). Tensile strength (TS) and elongation at break (EB) exhibited a similar change when increased chitosan and microfluidization pretreatment. The water vapor permeability (WVP) of the film decreased with increasing chitosan. The microfluidization pretreatment did not cause obvious change in WVP. Scanning electron microscopic (SEM) revealed exterior differences of the blend films. Thus, these results indicated that MPI films can used as a food packaging material in food industry.

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Key word: Mung bean protein isolates, Chitosan, microfluidization, Mechanical properties

1. Introduction

Biomaterial food packaging films substituted for synthetic polymer-based materials is a trend in food industry [1]. Protein is an important material can be used in food packaging film. Protein-based packaging attractive for applications including improving environmental protection, consumer health, and extending a product's shelf life. Protein-based films or coatings may be more interesting attribute to their nutritional value and gas barrier properties [2]. In general, proteins and polysaccharides tend to form stronger films with good gas barrier properties, renewable nature, and ability to degrade but are poor moisture barriers [3-6].

Mung bean (*Phaseolus aureus*) is increasingly becoming a more important leguminous crop and widely planted in Asia, Africa and America. Mung bean protein isolate is a vicilin-rich protein, and it is like 7s globulin [7-9]. Protein films are formed through the film forming solution carefully, and then by casting and drying at a constant relative humidity [10]. However, protein packaging has shown some limitations, i.e., resistance, water barrier function, mechanical properties, and high costs [11]. The aim of this research was to prepare an edible mung bean protein film and to evaluate the effects of chitosan and microfluidization pretreatment on mechanical properties of these protein-based films.

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2. Materials and Methods

2.1. Materials

Mung beans were purchased in a supermarket in Guangzhou of China, and were cultivated in Henan Province of China. Protein MW marker kit was from Dingguo Biol. Co. (China). All other chemicals were of analytical or better grade.

2.2. Preparation of mung bean protein isolates and microfluidization pretreatment

The flours and mung bean protein isolates (MPI) were prepared according to the method of El-Adawy, with some modifications [12]. Stock MPI solution (5%, w/v) was prepared by dissolving the native MPI in deionized water, and the pH was adjusted to 7.0. MPI solutions were subjected to the microfluidization treatment at a specific pressure level of 18,000 psi (about 124 MPa), in a Microfluidizer processor model M-110EH (Microfluidics, Newton, MA, USA). All the protein samples were passed though the equipment duplicate.

2.3 Film preparations

MPI was used as a base component in film forming solutions. These films were prepared by solution casting at pH 3.0. Chitosan solution (1 wt%) was prepared by dispersing the chitosan powder in 0.1 M lactic acid, and stirring about 12 h. MPI solution (5 wt%) dispersed in de-ionized water by stirring continuously 12 h at room temperature. Glycerol (4:10 w/w) was added to MPI and polymer blend solution, respectively. The sample solution: MPI (no CH added), MPI-Microfluidization (MPI-M), MPI-CH, and MPI-Microfluidization-CH (MPI-M-CH). Nisin was added to film-forming solution, and magnetically stirred for about 30 min. Finally, the film forming solution was cast onto a rimmed glass plates coated with polyethylene films (Clorox China Co. Ltd., Guangzhou, China). Each film was controlled at same casting and the length about 20 cm. The castings were air-dried overnight at room temperature. The casting were removed to a thermostatic chamber, and followed set at 55±3% relative humidity (RH) and 22±2 °C for 48 h. The films were cut into a predetermined size prior to testing.

2.4 Measurement of differential scanning calorimetry (DSC)

The thermal denaturation of MPI samples were examined using a TA Q100-DSC thermal analyzer (TA Instruments, New Castle, DE, USA). The procedure was that of Meng and Ma, with some modifications [13]. About 2.5 mg protein was accurately weighed into aluminum pan, and 10 mL of 0.05M phosphate buffer (pH 7.0) was added. The MPI samples and buffers were equilibrated at 25 ºC about 6 h. These pans were hermetically impacted and heated from 30 to 120 ºC at a rate of 5 ºC/min. A sealed empty pan was used as a reference. Onset temperature (T_m), peak transition or denaturation temperature (T_d), enthalpy of denaturation (DH) and cooperativity, represented by the width at half-peak height ($DT_{1/2}$), were computed from the thermograms by the Universal Analysis 2000, Version 4.1D (TA Instruments-Waters LLC). All experiments were conducted three times.

2.5 Particle and size analysis

The size distribution of film forming solutions manipulated using a Mastersizer 3000 (Malvern Instruments, Malvern, UK). Film forming solutions were dispersed into the Hydro 3000s

wet dispersion accessory. The samples are placed in a length of 1 cm cuvette. It is performed at a particle-sizing cell by using backscattering technology. In all cases, the samples were repeated three times.

2.6 Determination of film thickness (FT)

The FT were measured at five random positions using a micrometer (TAIHAI apparatus Co. Ltd., Shanghai, China), and the mean value was used to calculate film tensile strength.

2.7 Measurement of water vapor permeability (WVP)

The water vapor permeability of the films was determined at 25 °C and 50% RH [14]. A 20 mL cup was filled up to 1 cm of the top with distilled water and was covered with a sample protein film. The weight loss of the cup was measured over time, and the slope was calculated using linear regression analysis. The WVP ($\times 10^{-9}$ g m/m^2 s Pa) was then calculated using the following formula:

$$WVP = (WVTR \times L) / \Delta p$$

where the water vapor transmission rate (WVTR) is calculated by dividing the slope by the open area of the cup, L is the mean film thickness, and Δp is the corrected partial vapor pressure difference across the film specimen.

2.8 Measurement of tensile strength (TS) and elongation at break (EB)

The films′ TS and EB were determined using a TA-XT plus texture analyzer (Stable Micro Systems, London, U.K.). TS were measured at the maximum load of a sample by the initial representative section. EB were measured as the present change from the initial length to the point of sample failure [15].

2.9 SEM analysis

Micrographs of the MPI films samples were observed using a scanning electron microscope (FEI Philips XL30 SFEG). The films were fixed and then coated with a fine gold layer before SEM observation. All the samples were examined using an accelerating voltage of 15 kV.

2.10 Statistics

Microcal Origin V.7.0 software (OriginLab Co., Northampton, USA) was used for significant difference analysis.

3. Result and discussion

3.1 Thermal denaturation temperature and enthalpy value

Thermal characteristics of MPI were carried out using a differential scanning calorimeter [16]. Thermal transition properties of MPI and MPI-M present a broad endothermic peak. Table 1 lists the T_d data of the MPI sample, as well as MPI-M.

Table 1 Thermal transition properties of MPI and MPI-M

	T_m (ºC)	T_d (ºC)	ΔH ($J \cdot g^{-1}$)
MPI	67.29±0.63	70.52±0.57	4.37±0.27
MPI-M	67.85±0.46	72.34±0.50	4.05±0.38

Each data is the mean and standard deviation of triplicate measurements.

A major endothermic peak with denaturation temperature (T_d) of 70.52 ºC and enthalpy value (ΔH) of 4.37 J/g was observed for MPI. The sample treated with microfluidization exhibits that a significant increase in T_d, while the value of ΔH is nearly unaffected in MPI-M (Table 1). It is proposed that the smaller protein particles through microfluidization compared to the control. As the globules were smaller post microfluidization, more surface area for the protein molecules can promote to adsorb. In addition, in the case of MPI-M, there is a slight feeble thermal transition signal, probably suggesting the disruption of ordered protein structure. This data may further support the viewpoint that microfluidization protects the native structure of MPI.

3.2 Size distribution

The size distribution of the film forming solution was examined as a function of physicochemical properties. Size distribution of the film forming solution affected significantly by chitosan and microfluidization. Fig. 1 shows that the sample solution revealed significantly a trimodal size distribution. A trimodal size distribution composed smaller peaks (about 0.3 μm), medium peaks (about 10 μm), and larger ones (about 100 μm). As an essential protein-based film, the trimodal distribution is presented different solutions might not conducive stability of films system. In contrast, the globules of MPI treated with microfluidization became smaller compared to do not post microfluidization.

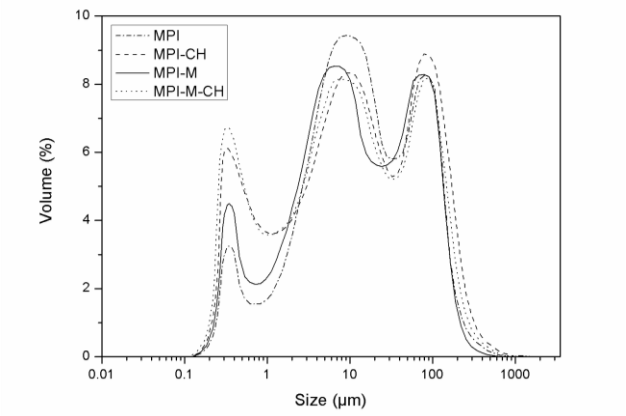

Fig. 1 Size distribution of MPI-based film forming solution

Although the trimodal peaks were observed, it is presumed that the smallest of these droplets, that with a size of less than 1 μm, was considered as protein aggregates and not protein coated chitosan droplets. The trimodal nature of the film forming solutions is attributes to the coalescence of smaller droplets enter into larger ones [17].

3.3 Mechanical properties of films

The mechanical properties (FT, TS, EB, and WVP) of MPI films as function of chitosan and microfluidization were examined. Chitosan is a cationic polysaccharide, which be considered as a film-forming solution for improving the physicochemical properties of these protein films [18]. The effect of chitosan-protein blend films exhibits more tensile strength and elongation at break than without chitosan. The complex between MPI and CH produced less rigid and more flexible films, which attributes microstructure of films boost up the tensile properties of MPI-CH composite films [19]. The MPI films pretreated by microfluidization exhibited an intensified behavior, which the composite films were less rigid and much more flexible. The microfluidization led to an increase in film TS and EB, due to the homogeneous inclusion caused molecules rearrangement more ordered.

Water vapour permeability (WVP) for MPI films as a function of chitosan is given in Table 2. The WVP values of MPI films reflect the degree of affinity between chitosan-protein complex and water molecules. The addition of chitosan can improve hydrophobic inclusions within the film. These can reduce water diffusion through the film. The microfluidization make protein aggregation or/and flocculation more small, and the microstructure exhibit more homogeneous. However, the value of WVP did not reveal significant difference in statistical analysis.

Table 2 Physical properties of the MPI films at various conditions

	FT (mm)	TS (MPa)	EB (%)	WVP ($\times 10^{-9}$g/m^{2}s Pa)
MPI	0.73±0.08b	2.12±0.36c	5.56±0.69d	3.43±0.43a
MPI-CH	0.98±0.11a	3.50±0.51ab	16.37±1.18b	3.27±0.55a
MPI-M	0.68±0.12b	3.38±0.46b	12.09±1.30c	3.19±0.32a
MPI-M-CH	1.07±0.17a	4.07±0.62a	36.82±4.21a	3.26±0.57a

a-c values in a column followed by diferent superscript letters are significantly different (p<0.05).

3.4 Films microstructure

SEM photographs analysis were carried out for insight into the microstructure of the protein films. Fig. 2 show that the surfaces structure of MPI-based films. All surfaces of MPI films reveal dense and slightly heterogenous structure. The SEM photographs observations strongly indicated that these composite protein films have significant differences in surface structure depending on the component and microfluidization pretreatment. Chitosan-protein blend films have been proved to be homogenous due to the good miscibility, and can improved film mechanical properties [20]. However, heterogenous surface of the films possibly due to the interruption of the protein network (Fig.2 b and d). Complex polymer and aggregation may be formed in the processing, which changed the structural characteristics. Compared to the films contain chitosan, both the MPI and microfluidization pretreatment films are rather homogenous and continuous. Unexpectedly, microfluidization pretreatment make the film looks like more delicate. In addition, the differences of TS and EB also reflected that the microstructure of MPI molecules rearrangement by post microfluidization.

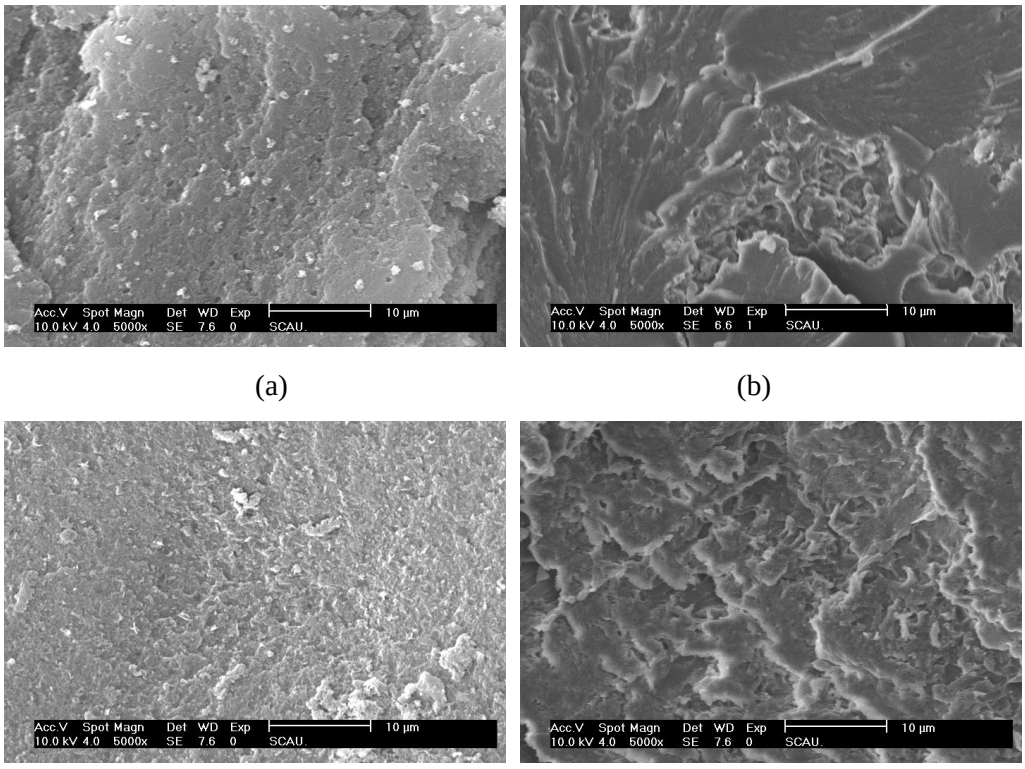

Fig.2 Surface morphology of MPI films by SEM. a) MPI, b) MPI-CH, c) MPI-M, d) MPI-M-CH.

4. Conclusions

Mung bean protein isolates can be used for packing film and it has excellent physical and mechanical properties. Addition of chitosan can make the film more flexible, thus, the mechanical properties of films increased in this processing. The microfluidization pretreatment change the film microstructure by rearranged protein molecule, and increased in TS and EB of the films. SEM analyses indicated that the external surface structure of the films, and reflected the mechanical and WVP properties. These results indicated that MPI films can used as a food packaging material in food industry.

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