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Effect of oxidization and chitosan on the surface activity of soy protein isolate

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ABSTRACT

The objective of this research was to study the effect of oxidization of performic acid and chitosan on the structure and surface properties of soy protein isolate. As the degree of oxidization increased, the emulsifying capacity and stability of all the oxidized soy protein isolate and chitosan (SPI/CHI) systems increased substantially, which were 29.7%, 31.7%, 34.1%, 31.9% and 31.9% respectively compared. Fluorescent spectrum showed that the fluorescence intensity of SPI/CHI conjugates decreased and the higher the oxidized degree was, the lower the fluorescence intensity. Results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that the location of acidic bands of SPI/CHI conjugates moved upwards and broadened. Meanwhile, the basic bands lightened or even disappeared gradually as the oxidization increased. Scanning electron microscope (SEM) showed that the particles became lager as the degree of oxidization increased. Better thermostability of the oxidized SPI/CHI systems was shown in the differential scanning calorimetry (DSC).

1. Introduction

Development of biodegradable polymers from renewable resources to replace conventional synthetic plastic products provides opportunities for reducing waste through biological recycling to the bio system. Biodegradable polymers based on cellulose, starch, protein, microbial polyesters and polylactic acid each have novel properties, which suggest possible applications (Nanda, Rao, Kar, & Nayak, 2007).

Soy protein is globular, reactive and often water soluble as compared to helical or planar, non-reactive and water resistant synthetic polymers (Kisku, & Swain, 2012). Soy protein consists of various polar and reactive amino acids such as cysteine, arginine, lysine and histidine which can be used for crosslinking it and improving the tensile and thermal properties (Chabba, Matthews, & Netravali, 2005). Soy proteins have commonly been used for food and animal feed for many years (Nanda, Rao, Kar & Nayak, 2007). Nowadays soy protein is used in many fields such as degradable films and plastics. Chitosan was used in many fields as a kind of renewable resources with cellular compatibility and degradability such as cosmic industry, food industry and medicinal industry (Ana, Verica, Sven, & Vladimir, 2015; Inês, Ana, Mariana, Inês, & Celso, 2013; Li, Dou, Fu, & Qin, 2015). Some researchers studied the conjugate of SPI and chitosan and used it as food resources (Yang et al., 2015). The traditional Maillard reaction by dry heating usually takes a long reaction time up to several days or weeks, and the reaction extent is relatively hard to control (Zhu, Damodaran, & Lucey, 2008). The main usage of soy protein lies in production of gel, adhesive, plastics and food additives. Soy protein has hydrophilic and hydrophobic groups at the same meaning the potential of surface activity like the traditional surfactant. But there were few study of this application of soy protein. The main modifying methods of soy protein were limited by the tense globular structure of protein molecules pertaining to the existence of disulfide bonds.

The aim of this work is to explore and improve the surface activities of soy protein by cleaving disulfide bonds in it with performic acid and then grafting with chitosan and to study the relation between structure of the oxidized SPI/CHI systems and corresponding surface activity. As a macromolecule, the reactive residues were wrapped inner soy protein, so traditional glycosylation with

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chitosan only scratch the surface of soy protein, which resulted in the limitation of modification. The cleavage of disulfide bonds at different degrees achieve different unfolding of soy protein and different exposure of reactive residues to polar environment, which makes it easier for chitosan to graft into the soy protein molecules. The higher the cleaving degree is, the more the chitosan graft into soy protein. Chitosan with abundant hydroxyl grafting into soy protein makes the balance between hydrophilic and hydrophobic groups more stable and more like the mechanism of traditional surfactant.

All the determinations of different samples showed that oxidized SPI/CHI systems are of better emulsifying capacity and stability, compared with the pure SPI/CHI. Including the antibacterial and fungus proofing properties of chitosan, the SPI/CHI conjugates have more promising characteristics than SPI or chitosan at the same time, which is of great significance for the theory of the natural protein-based surfactant. The degradable, eco-friendly, cheap and convenient protein-based surfactant means a lot to the environmental protection and the effective utilization of natural resources. The cleavage of disulfide bonds in soy protein isolates, which is a new perspective of modification of soy protein.

2. Materials and methods

2.1. Materials and instruments

2.1.1. Materials

Soy Protein Isolate (Henan Kun Hua Biological Technology Co. Ltd.); Chitosan (Shanghai Ka Bo Industry and Trade Co. Ltd.); Hydrogen peroxide (88%, AR, Xi Long Chemical Co. Ltd.); Formic acid (30%, AR, Xi Long Chemical Co. Ltd.).

2.1.2. Instruments

Avanti Centrifuge (J-26 XPI, the United States); High speed shear (100LX, China); Fluorescent spectrophotometer (F-320, Shimadzu, Japan); Electrophoresis apparatus (Bio-RADAE8800, United States); Scanning electron microscopy (S-3400N, Japan); Differential scanning calorimeter (STA449C; Netzsch); Malvern Zetasizer: (Nano ZS90, England).

2.2. Preparation of samples

2.2.1. Oxidization of soy protein isolate with different concentration of performic acid

Soy protein flour and deionized water were mixed at a 1:15 mass ratio, the pH of the solution was adjusted to 7.0, and then the solution was incubated at 45 °C for two hours. Next, the solution was centrifuged at 3500 r/min for 30 min. The supernatant was removed and the pH was adjusted to 4.6, and then refrigerated for two hours at 4 °C. Next, the solution was, centrifuged at 3500 r/min for 30 min, the supernatant liquid was discarded, and the remaining material was added to 1200 mL deionized water.

Prepare soy protein isolate solution (12 mg/mL, 100 mL) and number them from 1 to 6, performic acid (0, 1, 1.5, 2, 2.5, 3 mL) were added into soy protein solution, then the six samples were put in dark environment at −4 °C for 3 h 2.5 mL ethanol was put into six flasks respectively to end the reaction.

2.2.2. Preparation of SPI/CHI based on different oxidization

Chitosan hydration solution 1/100 (w/v, g/100 mL) were agitated for 3 h, and then was added into the oxidized SPI flasks, and the six mixed systems were agitated at 40 °C for 3 h. The concentration of the chitosan is 5 mg/mL in the final samples.

2.3. Surface properties of different samples

2.3.1. Emulsifying capacity and stability

The emulsifying capacity and stability were measured as described previously. To do this, 20 mL soy oil, 5 mL soy protein solution, and 25 mL deionized water were combined and put under the high speed shear at 10000r/min for 1 min. Next, the sample was put into a graduated cylinder and the height of the oil layer was measured after one hour, which corresponded to the emulsification height. The higher the emulsification layer, the better the emulsification capacity (EC) is.

$$\text{The formula is} \quad \text{EC}\,\% = \frac{\text{height of oil layer}}{\text{total height}} \times 100 \tag{1}$$

To determine the emulsification stability: apply the six different measuring cylinders into the water bath at 80 °C for 30 min. Waiting an hour after the temperature decreasing to room temperature, determined the height of oil layer, then the emulsification stability (ES) is:

$$\text{ES}\,\% = \frac{\text{height of oil layer bathed in hot water}}{\text{initial height of oil layer}} \times 100 \tag{2}$$

2.3.2. Foaming capacity and stability

According to the methods of (Hu, Huang, & Li, 2004), we added up 5 mL protein solution and 45 mL deionized water into a beaker, in which the intensity of soy protein was 2%, respectively. Put the mixed solution under the high speed shear at 10000r/min for 1 min. Write down the volume of the foam right after the shearing, then the foaming capacity (FC) formula is:

$$\text{FC}\% = \frac{\text{initial volume of foam}}{50} \times 100 \tag{3}$$

Write down the volume of foam 30 min after the shearing, and the foaming stability (FS) formula is:

$$\text{FS}\% = \frac{\text{volume of foam after 30 min}}{\text{initial volume of foam}} \times 100 \tag{4}$$

2.3.3. Determination of particle size

The concentration of samples is 12 mg/mL, the determination of particle size is operated on NanoZS90.

2.4. Structural properties of different samples

2.4.1. Fluorescent spectrum

We determined the inner fluorescence of different samples, at an excitation wavelength of 290 nm, and the exterior fluorescence of different samples using ANS (1-anilinonaphthalene-8-sulfonic acid ammonium) as a probe, excitation wavelength of 350 nm. The concentration of the samples were 6 mg/mL, and the excitation slit was 5 nm and the emission slit was 3 nm.

2.4.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Samples were separated on by SDS-PAGE using a 13% separation gel and 5% condensed gel. The applied volume of sample was 15 μL. Voltage during the condensed gel was 90 V and during the separate gel was 120 V. Following electrophoresis, the gel was stained using Commassie Brilliant Blue.

2.4.3. Scanning electron microscopy (SEM)

SEM was performed on a scanning electron microscopy to provide visual information of the top surface morphology. To acquire superb SEM images, all samples of the electrospun fiber films were placed on an aluminum tray and coated with gold to make the fiber films conductive.