Preparation and Applications of Soybean Residue CNF Films

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Abstract: In this study, soybean residues were treated with HCl and soybean residue cellulose was extracted, which was used to prepare cellulose nanofiber (CNF) using the high-pressure homogenization method. The maximum yield of CNF, the reaction temperature, reaction time, and HCl concentration were optimized. The optimum HCl concentration for acid treatment was 6%, the reaction time was 60 min, the reaction temperature was 80℃, and the maximum yield of soybean residue cellulose was 78.8%. The different CNF films were then prepared; the color, mechanical property, and light transmittance of the CNF films were studied. Compared to the properties of the CNF film prepared with the soybean residue cellulose by high-pressure homogenization 15 times (HGT-15 film), the mechanical properties of the CNF film with soybean residue cellulose by decolorizing treatment decreased, but the light transmittance increased. The film prepared by adding HGT-15 CNF to whey protein was investigated for its mechanical property, light transmittance, and solubility. Unlike the pure whey protein film, addition of 2.0% CNF to the whey protein enhanced the mechanical property and water vapor transmission rate (WVT) of the film. With the increase in CNF content, the solubility of the whey protein film decreased, and then stabilized.

Keywords: acid treatment; soybean residues; CNF; whey protein film

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1 Introduction

Cellulose nanofiber (CNF), also called nano-fibrillar cellulose, is one-dimensional <100 nm in size, and filamentous[1-2]. Furthermore, CNF has a high aspect ratio, high specific surface area, high mechanical strength, special optical and hydrophilic properties, excellent biocompatibility and biodegradability, making it suitable for application in paper, medicine, food, hygiene and absorbent products[3]. Soybean residue (SR), an agricultural waste, is an abundantly available, low cost, and renewable resource[4]. At present, only a small part of soybean residue can be fully used while most of it is used for feed, or is abandoned, causing environmental pollution and wastage of resources[5]. Soybean residue is the parenchyma tissue; the cell walls are thin, and the bonding force between the microfibrils is weak. Therefore, it has been used to produce CNF by high-pressure homogenization[6].

Whey protein (WP) is a milk protein that does not contain casein. It is a by-product of cheese processing, and mainly contains β-lactoglobulin and α-lactalbumin[7]. At present, whey protein is widely used in whey protein concentrate (WPC) and whey protein isolates (WPI). Since whey protein has a large number of hydrophilic groups on the surface, excellent emulsion stability, low cost, and is easily degradable, it has extensive application prospects in the food industry such as preparation of edible film[8]. Pure WP film has superior gas barrier properties, excellent elongation at break, and elasticity, but a low tensile strength[9].

In this study, soybean residue cellulose was extracted with HCl treatment; maximum yield of soybean residue cellulose was obtained by optimizing the reaction temperature, reaction time, and HCl concentration. The CNF was made into films after two different decolorizing methods, and then, analyzed for their color, mechanical property, and light transmittance. Finally, the mechanical property, light transmittance, and solubility of the film prepared by adding CNF to whey protein were investigated.

2 Experimental

2.1 Materials

Soybean residues were supplied by a soymilk mill (Heilongjiang, China). They were passed through a 40-mesh screen, and then, dried to 4.6% moisture content. Sodium hydroxide, sodium chlorite, glacial acetic acid, carboxymethyl cellulose (CMC), and glycerol ($C_3H_8O_3$) were purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Analytical grade HCl (36 wt%~38 wt%) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). The whey protein concentrate was purchased from Zhengzhou Yuzheng Food Additive Co., Ltd. (Zhengzhou, China).

2.2 Preparation of the CNF film

2.2.1 Extraction of soybean residue cellulose

2 g of soybean residues were extracted with petroleum ether at 50℃ for 12 h to remove the fat, followed by oven-drying at 55℃ for 24 h. The protein in the soybean residues was removed with 1.5% (*w/w*) protease at 50℃ for 2 h, and then, inactivated in a boiling water bath for 10 min.

For optimizing the HCl concentration, reaction temperature, and reaction time to obtain the maximum yield of soybean residue cellulose, the soybean residues were placed in various concentrations of HCl solutions and stirred in a water bath set for different reaction time at different temperatures. The resultant residue was continuously washed with deionized water until neutral, and the soybean residue cellulose samples were collected and freeze-dried.

2.2.2 Preparation of CNF

The soybean residue cellulose samples were homogenized using a high-pressure homogenizer (Xigao, China) operated at 60 MPa. The suspension consistency was 1%~2% during homogenization process. The HGT-15 CNF was collected after 15 passes and freeze-dried.

2.2.3 Decolorization

After the soybean residue is treated with HCl, the lignin in the soybean residue cellulose was further removed

by the following two decolorizing methods.

(1) Sodium chlorite method (SC method): 65 mL distilled water, 0.5 mL glacial acetic acid, and 0.6 g sodium chlorite were added to 2 g soybean residue cellulose at 75℃ for 1 h. After the completion of the reaction, the drugs (0.5 mL glacial acetic acid and 0.6 g sodium chlorite) were added 3 times, and then, the reaction mixture was cooled down to room temperature, suction filtered with a Buchner funnel, and washed repeatedly until the filtrate was neutral. The obtained samples were collected and were prepared into CNF (SC-15 CNF) by the method in Section 2.2.2.

(2) Acid-Base combination method (A-B method): 40 mL of 4% NaOH solution was added to 2 g soybean residue cellulose to remove the fat and protein at 60℃ for 1 h. After the completion of the reaction, the reaction mixture was suction filtered with a Buchner funnel and washed repeatedly until the filtrate was neutral. Then, the samples were added to an Erlenmeyer flask containing 8% HCl solution and kept at 75℃ for 80 min. The obtained samples were washed, suction filtered, collected, and were prepared into CNF (A-B-15 CNF) by the method in Section 2.2.2.

2.2.4 Preparation of CNF film

The CNF film was prepared by percolation drying method. The CNF obtained by high-pressure homogenization treatment was diluted to 2%, stirred, and suction filtered using a solvent filter with a 0.45 μm microfiltration membrane (1000 mL, Heqi Chemical Technology Co., Ltd., China). After filtration, the filter membrane was taken out and a clean filter membrane was placed on the surface of the filter membrane, which was then placed in the middle of two pieces of clean filter papers and pressed with a glass plate, then dried at 60℃ for 48 h, and then, the CNF film was peeled off. Finally, the film was placed in a sealed bag and collected and the prepared films are respectively recorded as HGT-15 film, SC-15 film and A-B-15 film.

2.2.5 Preparation of whey protein film

The whey protein film preparation is shown in Fig.1.

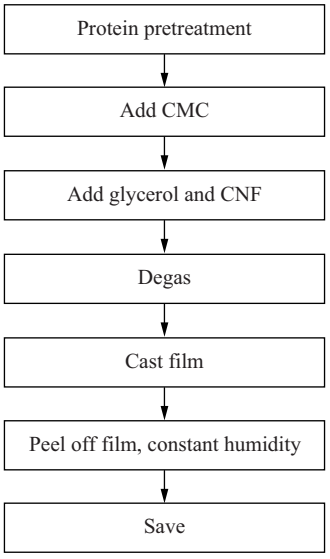

Fig.1 Flow diagram for the preparation of whey protein film

Deionized water (90 mL) was added to 10 g whey protein powder, and the solution was washed with 1% NaOH solution until pH value was 8. It was then put in a constant temperature water bath at 80℃ for 30 min, and stirred slowly and a small amount of 0.35% thickener (CMC) was added. After the CMC was completely dissolved, the solution was cooled down to room temperature, and 5 mL of plasticizer (glycerol) and a certain amount of CNF were added. Then it was stirred and degassed with ultrasonic cleaner for 4 h. Finally, the solution was placed in a film-forming device and dried at 60℃, and the film was peeled off and placed in a sealed bag for 48 h, then collected.

2.3 Structural characterization

2.3.1 Yield of soybean residue cellulose

The yield of soybean residue cellulose was calculated according to Eq. (1):

$$\text{Yield}\ (\%)=\frac{w_1}{w_2}\times 100\% \quad (1)$$

Where w_1 is the quality of soybean residue after HCl treatment, i.e, soybean residue cellulose, g; and w_2 is the quality of raw soybean residue, g.

2.3.2 Degree of polymerization of CNF

The average degree of polymerization (DP) of the CNF was measured by the copper ethylenediamine viscosity method. The 0.1 g of dry CNF sample, 25 mL distilled

water, 25 mL copper ethylenediamine solution, and 2~3 pieces of copper sheet were added to the sample bottle and stirred repeatedly until the sample was completely dissolved. The DP of CNF was calculated by measuring the viscosity of the solution and using Eq.(2):

$$DP^{0.905}=0.75\eta \quad (2)$$

Where DP is the average degree of polymerization of CNF, and η is the viscosity of the solution.

2.3.3 Mechanical property of the film

The mechanical property of the film was determined by the Servo multifunctional high and low temperature control testing machine (AI-7000-NGD, Gotwell, China). For testing the tensile strength, the film was cut into rectangles of 40 mm×5 mm, the stretching speed was set to 5 mm/min, and the gauge length between the two clamps was set at 15 mm.

2.3.4 Light transmittance of the film

The light transmittance of the film was measured between 200 nm and 800 nm wavelength by ultraviolet-visible-near-infrared (UV-NIR) spectrophotometer (Cary 5000, Agilent, America).

2.3.5 Thickness of the film

The thickness of the film was measured by the thickness gauge (313-A, Gotwell, China), determined using the average thickness of five different planes of the film.

2.3.6 Water vapor permeability of the film

Water vapor permeability (WVP) was determined by water vapor transmission rate (WVTR) tester (W3/060, Languang, China). The flat surface of the film, 73 mm in diameter, was placed in a moisture permeable cup with 10 mL of distilled water at 38℃ for 15 h and 90% relative humidity.

2.3.7 Solubility of the whey protein film

Distilled water (40 mL) was added to the dry film and stirred for 24 h, and then, filtered and dried at 60℃ to obtain a constant weight. The solubility was calculated according to Eq.(3):

$$G=\frac{w_3-(w_4-w_5)}{w_3}\times 100\% \quad (3)$$

Where G is solubility of film, %; w_3 is the dry weight of the film, g; w_4 is the dry weight of the insoluble of the film and filter paper, g; and w_5 is the dry weight of the filter paper, g.

3 Results and discussion

3.1 The components of soybean residues

The main components of soybean residues are listed in Table 1. Holocellulose and α-cellulose contents in soybean residues were relatively high. Lignin content in soybean residues was lower than that in thick-wall fiber raw materials, which provides a theoretical basis for the extraction of soybean residue cellulose, and also provides the possibility for the preparation of CNF. In addition, protein and fat contents in the soybean residues were also relatively high, and the presence of protein directly affects the purity of the soybean residue cellulose. Therefore, these impurities should be removed as much as possible in the process of extracting soybean residue.

3.2 Acid treatment of soybean residue

3.2.1 Effects of HCl concentration on soybean residue cellulose

The effects of HCl concentration on the yield and DP of soybean residue cellulose are shown in Fig.2, when the solid to liquid ratio of soybean residue and HCl was 1:20 (g/mL), the reaction temperature was 80℃ and the reaction time was 60 min.

As can be seen from Fig.1, the yield and DP of the soybean residue cellulose decreased with the increase in HCl concentration. When the HCl concentration was less than 6%, the degree of hydrolysis of the soybean residues increased with the increase of the

Table 1 Components of soybean residues %

Holocellulose	α-cellulose	Protein	Fat	Acid soluble lignin	Acid insoluble lignin	Ash
70.64	63.13	15.60	5.88	0.15	1.16	3.21

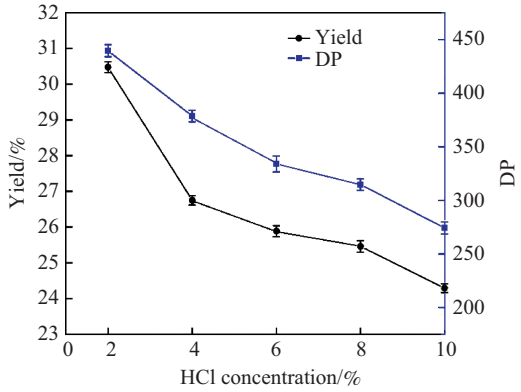

Fig.2 Effects of HCl concentration on the yield and DP of soybean residue cellulose

HCl concentration, therefore, the yield and DP of the soybean residue cellulose decreased; and when the HCl concentration was 6% to 8%, the yield and DP of soybean residue cellulose decreased slowly, mainly due to the denaturation of protein in the soybean residues, which slowed down the hydrolysis rate of the soybean residues. When the HCl concentration was greater than 8%, the yield and DP of soybean residue cellulose decreased rapidly, as the soybean residues cellulose was further hydrolyzed under the strong action of HCl. Therefore, the optimal concentration of HCl was 6%.

3.2.2 Effects of reaction time on soybean residue cellulose

The effects of reaction time on the yield and DP of soybean residue cellulose are shown in Fig.3, when the solid to liquid ratio of soybean residue and HCl was 1:20 (g/mL), the reaction temperature was 80℃, and the HCl concentration was 6%.

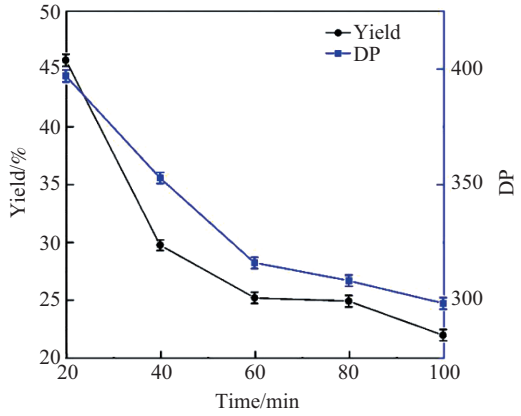

Fig.3 Effects of reaction time on the yield and DP of soybean residue cellulose

It can be seen from Fig.3, the yield and DP of soybean residue cellulose decreased gradually with the increase in reaction time. When the reaction time was >60 min, the yield and DP of soybean residue cellulose decreased slowly because the hydrolysis of soybean residues reached the maximum limit. Therefore, the optimal reaction time was 60 min.

3.2.3 Effects of reaction temperature on soybean residue cellulose

The effects of the reaction temperature on the yield and DP of soybean residue cellulose are shown in Fig.4, when the solid to liquid ratio of soybean residue and HCl was 1:20 (g/mL), the reaction time was 60 min, and the HCl concentration was 6%.

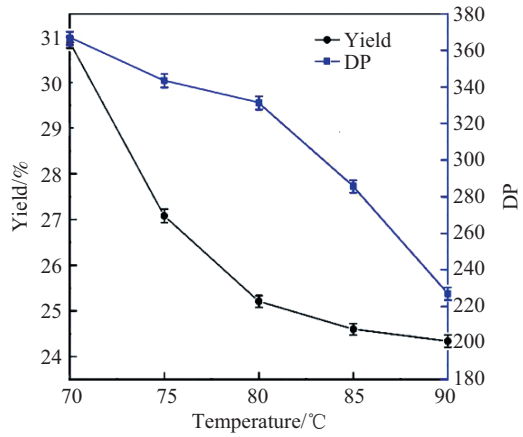

Fig.4 Effects of reaction temperature on the yield and DP of soybean residue cellulose

The yield and DP of the soybean residue cellulose decreased with the increase in reaction temperature as shown in Fig.4. The decrease was due to the hydrolysis of some cellulose at high temperature, but when the reaction temperature was >80℃, the yield of the soybean residue cellulose decreased slowly due to the denaturation of the protein in the soybean residues and the decreasing hydrolysis rate of soybean residues[10]. Therefore, the optimal reaction temperature was 80℃.

3.2.4 SEM analysis of CNF

The microstructures of soybean residue cellulose and HGT-15 CNF are shown in Fig.5(a) and Fig.5(b), respectively. Comparing Fig.5(a) and Fig.5(b), it can

be observed that the cell walls of both soybean residue cellulose and HGT-15 CNF were further damaged, and the morphology of soybean residue cellulose was noticeably altered. Fig.5(b) represents an evenly distributed nanoscale network structure of HGT-15 CNF, which confirms that the isolation of CNF from soybean residues with HCl treatment and high-pressure homogenization was successful.

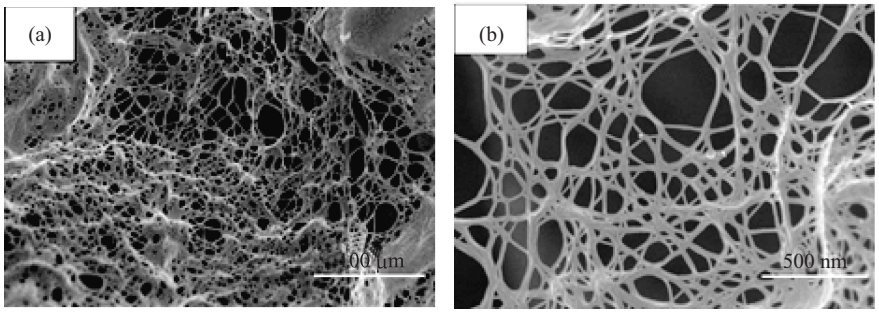

Fig.5 SEM images of soybean residue cellulose (a) and HGT-15 CNF (b)

3.3 Effect of decolorizing method on the CNF film property

3.3.1 Effect of decolorizing method on the CNF film color

Decolorization improves the sensory property of CNF. The CNF film was prepared by decolorizing the soybean residue cellulose with the SC method and the A-B method, and the pictures of different CNF films are shown in Fig.6.

HGT-15 film SC-15 film A-B-15 film

Fig.6 Effect of decolorizing methods on the CNF film color

After HCl treatment, most of the hemicellulose in the soybean residues were removed, the contents of acid-soluble and acid-insoluble lignin were comparatively less than those in the raw material. Since both the decolorizing methods destroy the chromophore of lignin, resulting in the removal of lignin. Therefore, the color of the HGT-15 film due to the presence of lignin was darker than that of the decolorized CNF films[8].

3.3.2 Effect of decolorizing methods on mechanical properties of the CNF films

In general, greater the density of the film, greater is the bonding force within the CNF films, imparting them better mechanical properties[11]. The effects of different decolorizing methods on the mechanical properties of CNF films are listed in Table 2.

Table 2 Effect of different decolorizing methods on mechanical properties of the CNF films

Samples	Basic weight /(g·m^{-2})	Thickness /μm	Elongation at break /%	Tensile strength /MPa	Elastic modulus /MPa
HGT-15	36	60	1.05	19.3	1836.2
SC-15	36	100	1.91	7.63	470.2
A-B-15	36	70	3.14	8.85	593.9

As can be seen from Table 2, under certain conditions, the thickness of the HGT-15 film was the least, and the thickness of the SC-15 film was the highest, but the corresponding tensile strength and elastic modulus of the two CNF films were opposite. The decolorizing methods mainly removed lignin. However, after the decolorizing treatment, the tensile strength of CNF film with decolorizing treatment was lower than that of the CNF film without the decolorizing treatment, indicating that the presence of lignin influences the mechanical properties of CNF film.

3.3.3 Effect of decolorizing methods on the light transmittance of the CNF films

The light transmittance of the CNF films is shown in Fig.7.

It can be observed from Fig.7, the light transmittance of the CNF films after decolorization was higher than

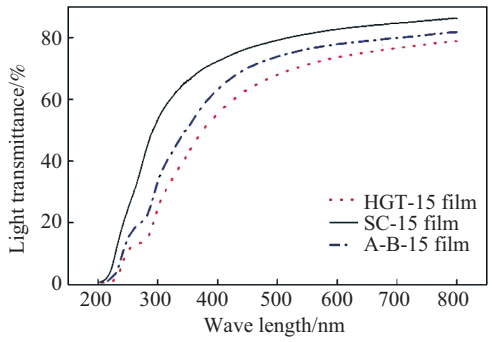

Fig.7 Effects of different decolorizing methods on the light transmittance of CNF films

that of the non-decolorized CNF film, and the light transmittance of SC-15 film was greater than A-B-15 film. The chromophoric substance in the HGT-15 film was mainly lignin. The carbonyl groups and conjugated double bonds in lignin could absorb ultraviolet light, making the light transmittance of SC-15 film greater than A-B-15 film and HGT-15 film. And the presence of lignin could prevent the separation of CNFs, increasing the binding force between CNFs and decreasing the light transmittance of CNF films.

3.4 Effect of CNF on the properties of whey protein film

Since biopolymers are cheap, renewable, and environmental-friendly resources, and have the film-forming property, they are suitable for the preparation of films. In particular, the whey protein films were studied. The whey protein film has good gas barrier properties, and excellent elongation at break and elasticity, but its tensile strength is low. Due to the excellent properties of CNF, it can be used as the reinforcing phase of composite materials to improve the strength of composite materials. Therefore, in this experiment, the effects of the mechanical properties, solubility, and water vapor transmission rate of whey protein film were investigated by adding different amounts of CNF to the whey protein[12].

3.4.1 Effect of the HGT-15 CNF content on the mechanical properties of the whey protein film

The effects CNF content on tensile strength and elongation at break of the whey protein film are shown in Fig.8.

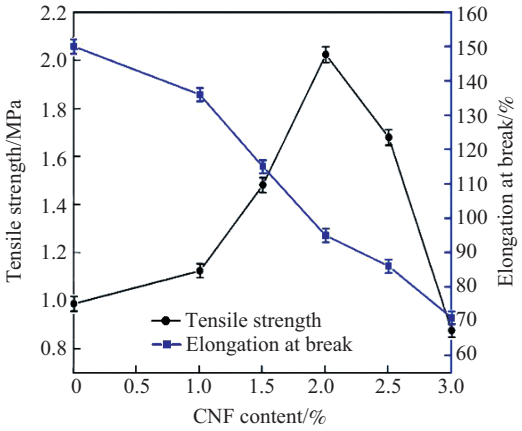

Fig.8 Effect of CNF content on tensile strength and elongation at break of the whey protein film

Fig.8 showed that when CNF content increased from 0 to 2%, the tensile strength of the whey protein film increased, but when CNF content was >2%, the tensile strength of the whey protein film decreased. This was due to the form of intermolecular hydrogen bonds between the hydroxyl group in the CNF and the amino group and the amide group in the whey protein. The internal structure of CNF in the whey protein film was responsible for skeleton strengthening, thus, increasing the tensile strength of the film. Further increase of the CNF content caused hindrance in its dispersion in whey protein and decreased the uniformity and the tensile strength of the film.

When CNF content increased, the elongation at break of the whey protein film decreased. This was due to the hydrogen bond formed by the interaction between whey protein and CNF and the van der Waals force between the CNF molecules destroyed the elasticity of the whey protein film[12-13]. Increase in CNF content led to its uneven dispersion in the whey protein, intertwined CNF, deterioration of the uniformity of the film, increased plasticity, and decreased ductility and elongation at break.

3.4.2 Effect of the CNF content on the water vapor transmission rate (WVTR) of the whey protein film

The effect of CNF content on the WVTR of whey protein film is shown in Fig.9. The WVTR of the whey protein film decreased with an increase in CNF content

(see Fig.9). When CNF content was low (<1.5%), it was evenly distributed in the whey protein, resulting in a dense crystallization of the CNF and slowing down of moisture diffusion, thus decreasing the WVTR of the film. However, when CNF content continuously increased (1.5%~2.0%), the interaction between CNFs enhanced, and the possibility of flocculation increased. There were gaps in the whey protein film that accelerated the diffusion rate of water vapor and the WVTR of whey protein film increased slowly[13]. If CNF content further increased (>2.0%), the CNF and the whey protein film tended to form a more dense network structure by hydrogen bonding, thus decreasing the diffusion path of water vapor, increasing the diffusion time of water molecules in the film and decreasing the WVTR of the film.

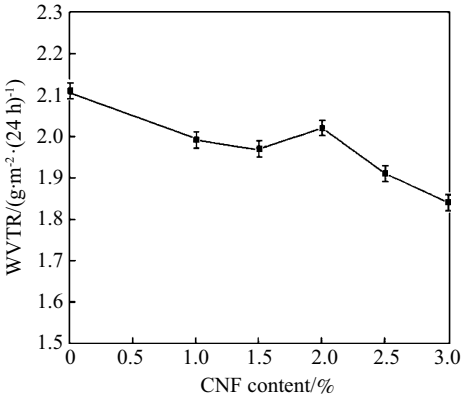

Fig.9 Effect of the CNF content on WVTR of the whey protein film

3.4.3 Effect of CNF content on the solubility of the whey protein film

The effect of CNF content on the solubility of the whey protein film is shown in Fig.10. With an increase in CNF content, the solubility of the whey protein film decreased and tended to become stable as shown in Fig.10. This was mainly due to the uniform dispersion of CNF when CNF content was low, which aided in the interaction between the CNF and whey protein network structure and increased the solubility of the whey protein in water. When CNF content was high (>2.5%), the interaction between the CNFs increased, which decreased the dispersion of CNF in whey protein. Therefore, the solubility of the film remained unchanged. In combination with various properties, the optimum amount of CNF added to whey protein was 2.0%.

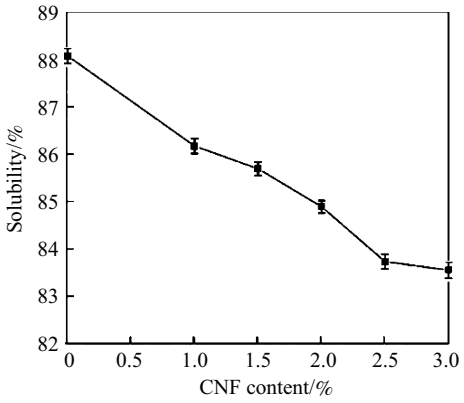

Fig.10 The effect of CNF content on the solubility of the whey protein film

4 Conclusions

In this study, soybean residue CNF was obtained after HCl treatment, two different decolorizing treatments and high-pressure homogenization; and different CNF films were prepared; and then, the mechanical properties, color, light transmittance of CNF films were explored. Finally, the mechanical property, light transmittance, and solubility of the film prepared by adding CNF to whey protein were studied.

(1) The optimum conditions for obtaining soybean residue cellulose by HCl treatment were: 6% HCl concentration, 60 min reaction time, and 80℃ reaction temperature, and the maximum yield of soybean residue cellulose was 78.8%.

(2) Removing the lignin from the CNF by the two different decolorizing methods, sodium chlorite (SC) method and acid-base (A-B) method changed the CNC films to light color. The mechanical property of the CNF film decreased after decolorization, whereas light transmittance increased. The light transmittance of the SC-15 film was greater than that of A-B-15 film.

(3) Addition of 2.0% CNF to the whey protein improved the mechanical property and water vapor transmission rate (WVTR) of the film, and the solubility of the film remained stable when the CNF content was >2.5%.

Acknowledgments

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