Effect of Secondary Structure determined by FTIR Spectra on Surface Hydrophobicity of Soybean Protein Isolate

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Abstract

The Fourier transform infrared spectroscopy (FTIR) was employed to study the relationship between secondary structure and surface hydrophobicity in different sorts of soy protein isolate. The results show that the surface hydrophobicity increases with the decrease of \(\alpha\)-helix content, and decreases with the increase of \(\beta\)-sheet and random coil content. There is no significant relationship between \(\beta\)-turn and the surface hydrophobicity.

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Key words: FTIR; soy protein isolate; surface hydrophobicity

1. Introduction

Soybean protein isolate (SPI) not only has high nutritional value, but also possesses a wide range of functional properties which play an important role in food processing, including solubility, hydration,
emulsification, foaming, gelation and aggregation [1-4]. The solubility of protein is one of important physical properties because of its restriction to other functional properties [5-8]. While solubility depends on the balance of hydrophilic and hydrophobic amino acids that make up the protein, Shigeru Hayakawa [9] found that hydrophobicity of soybean protein showed a negative correlation with solubility. Numerous studies show that the surface hydrophobicity of the protein significantly affect other functional properties of proteins [10,11], therefore, surface hydrophobicity of protein is a key measure of functional properties. Fourier transform infrared spectroscopy (FTIR) is a well-established tool in the determination of protein secondary structure [12,13]. In this research, The FTIR spectra was employed to study the relationship between secondary structure and surface hydrophobicity in different sorts of soybean protein isolate. This is helpful in maximizing the potential for functional properties of soybean protein isolate.

2. Methods

2.1. Materials

MAGNA-IR560 E.S.P-Fourier transform infrared spectroscopy system (U.S. Nicolet Company), DU800 UV spectrophotometer, F-4500 fluorescence spectrophotometer (Japan. Hitachi), High-speed low-temperature centrifuge (U.S. Beckman-Coulter company).

Different sorts of soybeans (Dongnong46, Heinong46, Wandou 34, Wuxing4, nf58, Zhonghuang13) were purchased from Chinese Academy of Agricultural Sciences, Lowry-method protein assay kit was purchased from Shanghai Labaide biotechnology CO.LTD.

2.2. Procedure

2.2.1 Preparation of soy protein isolate (SPI)

Soybean was cleaned and then dried in an electric oven at 45 °C for 12 h to a moisture content below 7%. The dried soybean was ground to 60 mesh and defatted with hexane at room temperature and allowed to dry in a fume hood by spreading the defatted soybean flour (DSF) in thin layers on an enamel plate.

DSF was suspended in deionized water in a liquid solid ratio of 15:1 (v/w) and adjusted to pH 8.0 with 2 mol/L NaOH, the mixture was stirred for 1.5 h then centrifuged at 10000g for 30 min. The supernatant was adjusted to pH 4.5 with 2 mol/L HCl and centrifuged at 6000g for 30 min. After washing with water and neutralized to pH 7.0 with 2 mol/L NaOH. All procedures were carried out at room temperature. The samples were freeze-dried and stored in cool place.

2.2.2 Determination of the nitrogen solubility index (NSI)

A 100 mg SPI sample was added to 10 ml of deionized water. The mixture was then stirred for 30 min at 20 °C, and centrifuged at 10000g for 20 min to obtain a supernatant and precipitate. The supernatants were appropriately diluted to determine protein content by Lowry method [14], the nitrogen levels were determined after filtration by the Kjeldahl method. The NSI was calculated as the percentage of the nitrogen extracted as to the total amount of nitrogen in the sample.

2.2.3 Determination of surface hydrophobicity of SPI
Surface hydrophobicity of SPI was determined using the hydrophobicity fluorescence probe ANS according to the method of Kato & Nakai [15]. A. Kato and S. Nakai, SPI samples were dissolved in 0.5 mg/ml in 0.01 M phosphate buffer pH 7.0, stirred for 1 h at room temperature, centrifuged at 10,000 g for 30 min and dispersible protein was determined in the supernatants. Each supernatant was serially diluted with the same buffer to obtain protein concentrations ranging from 0.005 to 0.5 mg/ml. Then, 30 µl of ANS (8.0 mM in 0.1 M phosphate buffer, pH 7) was added to 3 ml of the sample. Fluorescence intensity was measured with a F-4500 fluorescence spectrophotometer, at wavelengths of 338 nm (excitation) and 496 nm (emission). Each supernatant has been diluted with the same buffer to obtain protein concentrations ranging from 0.005 to 0.5 mg/ml was used as the control. The degree of surface hydrophobicity ($S_0$) was expressed as the ratio of fluorescence intensity of the SPI sample to that of the control.

2.2.4 Fourier-transform-infrared spectroscopy (FTIR)

The SPI samples were prepared with postassium bromide (KBr) pellet method. Infrared spectra were measured with a Magna-IR560 Fourier transform infrared spectrometer at 25 °C. For each spectrum 256 interferograms were collected with a resolution of 4 cm$^{-1}$ with 64 scans and a 2 cm$^{-1}$ interval from the 4000 to 400 cm$^{-1}$ region. The system was continuously purged with dry air. Second derivation spectra were obtained with Savitsky–Golay derivative function soft[16,17]. The relative amounts of different secondary structure of SPI were determined from the infrared second derivative amide spectra by manually computing the areas under the bands assigned to a particular substructure.

2.2.5 Statistical analysis

All determinations were carried out for three times. Means comparisons were made by Duncan's multiple range test. Data were analyzed by one-way analysis of variance (ANOVA) using the SPSS 17.0 computer program. A significant level was defined as a probability of 0.05 or less.

3. Results and discussion

3.1. The protein content of raw materials

Table 1. Protein content of different sorts of SPI

<table>
<thead>
<tr>
<th>Sort</th>
<th>Dongnong46</th>
<th>Heinong46</th>
<th>Wandou 34</th>
<th>Wuxing4</th>
<th>nf58</th>
<th>Zhonghuang13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (%)</td>
<td>94.2</td>
<td>96.6</td>
<td>94.5</td>
<td>93.7</td>
<td>95.4</td>
<td>95.8</td>
</tr>
</tbody>
</table>
3.2. The NSI and surface hydrophobicity of SPI

Fig. 1 shows that the NSI of SPI is affected by different sorts. The different sorts of SPI are ranked in order of NSI: nf58 > Zhonghuang13 > Wuxing4 > Wandou34 > Heinong46 > Dongnong46.

![Fig. 1 The NSI of different sorts of SPI](image1)

Fig. 2 indicates that there are differences on the surface hydrophobicity of SPI among different sorts. The descending order of surface hydrophobicity (S0) of six sorts of SPI is listed as follows: Dongnong46 > Wandou34 > Hongnong46 > Wuxing4 > Zhonghuang13 > nf58.

![Fig. 2 The surface hydrophobicity of different sorts of SPI](image2)
3.3. Comparison of FTIR spectra of six sorts of proteins

In order to study proteins, the analysis of the secondary structure of protein are often required by FTIR in recent years. The secondary structure of the protein were commonly based on the amide I band analysis (1700–1600 cm⁻¹). Amide I band peaks identified method is more mature now. Amide I is the most intense absorption band of the polypeptides. ν (C=O) has a predominant role in amide I, ν (C-N) follows. There are also some in-plane NH bending contribution to Amide I. The secondary structure of proteins are reflected by these bands as follows: 1610 ~ 1640 cm⁻¹ for the β-sheet; 1640 ~ 1650 cm⁻¹ for the random coil; 1650 ~ 1658 cm⁻¹ for the α-helix; 1660 ~ 1700 cm⁻¹ for the β-turn[18].

![Fig.3 FTIR spectra of different SPI](image1)

![Fig.4 The amide I region of different SPI](image2)

Fig.3 depicts the FTIR spectra in the wave number range of 4000 - 400 cm⁻¹ of six sorts of SPI, a strong absorption in the amide I band between 1700 and 1600 cm⁻¹ are indicated, which is shown in Fig.4. The original spectra in amide I band of different varieties of SPI are used to obtain the secondary derivative spectra. The secondary derivative spectra of different sorts of soybean protein isolate were fitted by the
Gaussian peak to show the characteristic peaks of the α-helix, β-sheet, β-turn and random coil. Then the peak areas are calculated to identify the secondary structure contents of different SPI, the results of contents are listed in Table 2. Table 2 shows that the descending order of α-helix content in different sorts of SPI is as follows: nf58 > Zhonghuang 13 > Wuxing 4 > Heinong 46 > Wandou 34 > Dongnong 46. Compared with the order of surface hydrophobicity, we finds that the lower α-helical content, the higher value of surface hydrophobicity. That is to say, the α-helix content and the value of surface hydrophobicity showed negative correlations; β-sheet and random coil content are ranked in order: Dongnong 46 > Wandou 34 > Heinong 46 > Wuxing 4 > Zhonghuang 13 > nf58, compared with the surface hydrophobicity, the higher β-sheet and random coil content, the bigger value of surface hydrophobicity, namely β-sheet and random coil content and surface hydrophobicity shows positive correlations; however, the linear relationship between β-turn content and surface hydrophobicity is not obvious. The probably reason is that the content of α-helix in protein is low and the protein molecular structure is relatively loosen when the β-sheet and random coil content is high, the hydrophobic sites in the protein are exposed largely, and then perform greater surface hydrophobicity.
Fig. 5 The amide I region of soybean protein isolate

Table 2 Content of the secondary structure in different varieties of soy protein isolate

<table>
<thead>
<tr>
<th></th>
<th>α-helix (%)</th>
<th>β-sheet (%)</th>
<th>β-turn (%)</th>
<th>Random coil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dn46</td>
<td>17.1</td>
<td>31.4</td>
<td>23.9</td>
<td>27.6</td>
</tr>
<tr>
<td>nf58</td>
<td>25.2</td>
<td>24.8</td>
<td>24.6</td>
<td>25.4</td>
</tr>
<tr>
<td>hn46</td>
<td>18.3</td>
<td>29.6</td>
<td>26.1</td>
<td>26.0</td>
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<tr>
<td>wd34</td>
<td>17.8</td>
<td>30.9</td>
<td>24.9</td>
<td>26.4</td>
</tr>
<tr>
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<td>20.4</td>
<td>28.7</td>
<td>25.1</td>
<td>25.8</td>
</tr>
<tr>
<td>zh13</td>
<td>21.4</td>
<td>25.5</td>
<td>27.6</td>
<td>25.5</td>
</tr>
</tbody>
</table>
4. Conclusion

The results show that the surface hydrophobicity increases with the decrease of α-helix content, and decreases with the increase of β-sheet and random coil content. There is no significant relationship between β-turn and the surface hydrophobicity.

Acknowledgements

The authors would like to thank the Heilongjiang Science and Technology Agency (research grant number: GA09B401-6), National Institute of Soybean Engineering Technology and Northeast Agricultural University for funding this work. We would also like to thank other students for their assistance.

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