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Study on secondary structure of hulless barley (*Hordeum Vulgare* L.) protein using FTIR and CD spectrum

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ABSTRACT

The main point of this work is to evaluate the the secondary structure of hulless barley protein for the research of its integrated utilization in the animal feed and food industries. The structure of hulless barley protein was observed with scan electron microscopy. The secondary structures of hulless barley protein were determined using FTIR and CD spectrum. The sizes of hulless barley protein were normally 20-60 microns (millionths of a meter) across. The mainly secondary structures of hulless barley protein were β-sheet (44.60 % and 48.27 %), β-turn (35.67 % and 36.47 %), and the α-helix (19.73 % and 18.15 %) obtained from the FTIR and CD, respectively. The results showed that the methods of the FTIR and CD could effectively detect the secondary structure of hulless barley proteins, and their structural features were spherical and relatively stable. © 2015 Trade Science Inc. - INDIA

KEYWORDS

Hulless barley protein;
Fourier tansform infrared spectrum (FTIR);
Circular dichroism spectrum (CD);
Secondary structure.

INTRODUCTION

Hulless Barley (*Hordeum Vulgare* L.) grows and looks like traditional barley until nearly mature. Compared to traditional barley, hulless barley is more attractive to the animal and poultry feeding industries because of higher total protein and digestible energy contents than hulled barley[1, 2].

In China, hulless barley is the main cereal crop in plateau areas with high altitudes from 2500 to 3000 meters in southwestern of China, in particular wide distribution among Tibet, Qinghai, and parts of Gansu, Yunnan and Sichuan Provinces[3], which produced in Qinghai-Tibet Plateau about 550,000-600,000 tons every year. In fact, in the other countries, such as, Canada, barley grain yield was around 800,000 tons in 1998[4]. However, the processing utilization degree of hulless barley is relatively low, mainly focuses on traditional brewing food, rough machining products and animal feed in the Tibetan compatriots. Its comprehensive utilization and research are still at the stage of enlightenment, which cannot form the food processing industry.

A Fourier transform infrared (FTIR) spectroscopy has emerged as a useful tool for the characterization of protein secondary structure[5]. The circular dichroism (CD) spectrum of a globular protein can be expressed as a linear equation[6]. The methods for correlating the CD spectra and the secondary structure of proteins have been developed in re-

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cent decades[7].

The main objectives of this study were to evaluate the the secondary structure of hulless barley protein for the research of its integrated utilization in the animal feed and food industries.

MATERIALS AND METHODS

Materials

Hulless barley (*Hordeum vulgare* L. subsp. *vulgare*) was kindly provided by Food Bureau of Qinghai Province. The hulless barley protein was pruified as described previously by Wang et al.[8]. The process is: material, socked in water, pulping, homogeneity, added cellulase, thermostatic mixing, filter, enzyme deactivation, centrifugation, washing, protein.

The protein structure analysis with scan electron microscopy (SEM)

The gold films of protein samples after the drying process have been coated by making use of ion beam sputtering deposition, and were observed under scan electron microscope.

The secondary structure of protein analysis with FTIR

IR spectra were recorded with an IR Prestige-21 (Shimadzu Corporation, Japan) Fourier transform spectrometer equipped with a liquid N_2-cooled mercury cadmium telluride detector. Protein samples, which freeze drying process, were inserted between CaF_2 windows using 1:100 (mass ratio). For all spectra, among the 400-4000 cm^{-1} has been normalized to unity. The second derivatives, used to resolve the overlapping bands with Gaussian, were calculated using the software of PeakFit v4.12.

The secondary structure of protein analysis with CD

The CD spectra were measured with a JASCO J-715 spectropolarimeter in a rectangular quartz cuvette of 0.1 cm path length. Repetitive scans (5–10 scans) were recorded and averaged at 25 °C with a 1 s integration time, a 0.1 nm step size, and a 1.5 nm bandwidth. Solvent spectra were subtracted from the measured spectra of protein. The wavelengths of scanning were 190-250 nm. The optical path of quartz sample pool was 0.1 cm. The resolution ratio was 0.2 nm. The sensitivity was 20 mdeg/cm. The method was determined as described by Corbin et al.[9]. The reported spectra were the average of eight scans. Secondary structure estimations were obtained by spectral deconvolution using the software of Selcon3 to calculate the proportion of secondary structures.

RESULTS

The analysis of the structure characteristic of hulless barley protein under SEM

Figure 1 shows scan electron micrograph of a sample in which the microscopic surface textures of hulless barley proteins, which their surfaces were irregularities, were bound up with their particle sizes. Flakes of material are present on the surface. The profiles of hulless barley proteins appear mostly intact, and were of roughly spheres, loose arrangement and certain gaps (Figure 1). There were great differences in size, which were normally 20-60 microns (millionths of a meter) across, and there were little stickiness each other.

The analysis of the secondary structure of hulless barley protein with FTIR

The absorption peak in 1660.8 cm^{-1} should be amide I region. The absorption peak in 1544.3 cm^{-1} should be amide II region, and in 1245.8 cm^{-1} amide III region (Figure 2). There are slightly different with the amide III absorption region of general protein (1300-1260 cm^{-1}). These results might be related to the nature of the hulless barley protein.

The amide I regions (1700-1600 cm^{-1}) of FTIR spectra of hulless barley protein along with FTIR spectral enhancement and second-derivative analysis had seven absorption peaks (Figure 3). According to the peak areas of branch fitting to calculate the corresponding to the proportion of secondary structure, the results showed in TABLE 1. The mainly secondary structure was β-sheet, which its content was about 44.60 %. The content of β-turn structure was about 35.67 %, and the content of α-helix was

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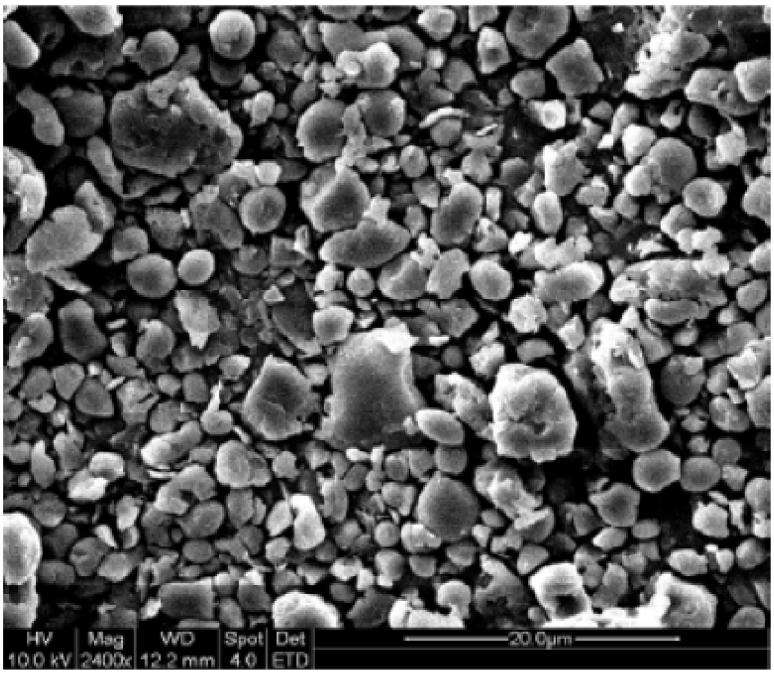

Figure 1 : Electron micrograph of hulless barley protein (Bar =200um)

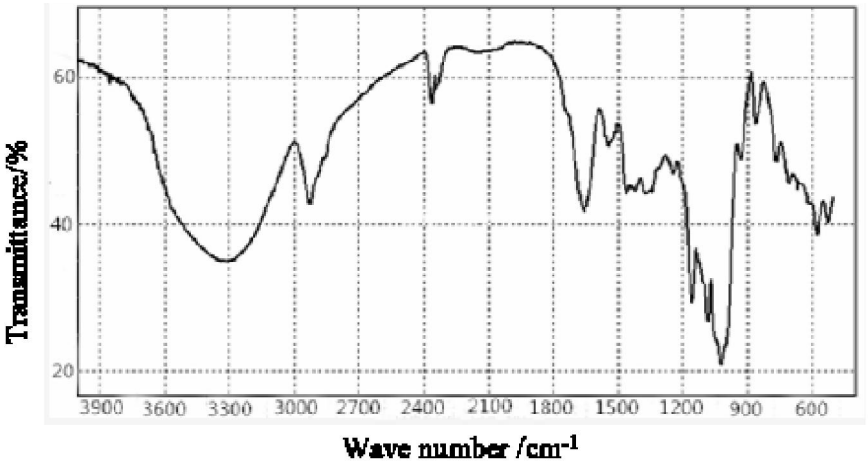

Figure 2 : FTIR spectra of hulless barley protein (4000-400 cm^{-1})

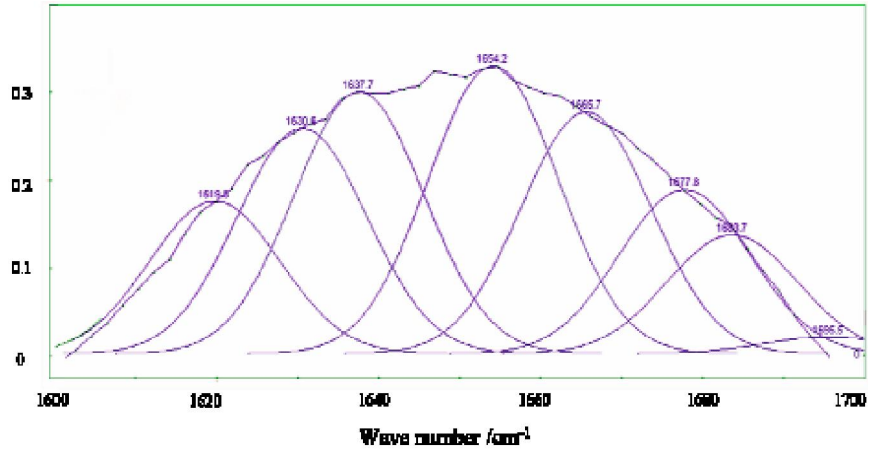

Figure 3 : Deconvolved amide‘!region of hulless barley protein with well-known secondary structures containing *alapha*- and *beta*-segments

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TABLE 1 : Secondary structure composition of hulless barley protein by FTIR spectra

Frequency/ cm^{-1}	The types of secondary structure	The contents of secondary structure / %
1619.8	β-sheet	
1630.6	β-sheet	44.60
1637.7	β-sheet	
1695.5	β-sheet	
1654.2	α-helix	19.73
1665.7	β-turn	
1677.8	β-turn	35.67
1683.7	β-turn	

TABLE 2 : Correlation between common protein structures and amide I frequency

Structure	Amide I frequency(cm^{-1})
Antiparallel β-sheet/Aggregated strands	1675-1695
3_{10}-Helix	1660-1670
α- Helix	1648-1660
Unordered	1640-1648
β-sheet	1625-1640
Aggregated strands	1610-1628

only 19.73 %.

The analysis of the secondary structure of hulless barley protein with CD

There were a positive absorption peak in 191 nm and a little negative peak in 209 nm in the far UV region (180-250 nm), which it was the structure characteristic peak of α-helix but there was not the other structure characteristic peak of α-helix in 222 nm. There are a negative band at near 217 nm and a positive peak in 194 nm, which it was the characteristic peak of β-sheets. Moreover, there is a characteristic peak of β-sheets in 205 nm.

The performance indices for each of the secondary structures in solution which calculated by analysis software packages related by JASCO J-715 CD, the content of regular β-sheet was the highest, about 48.27 %, followed the β-turn, about 36.47 %, and the regular α-helix, about only 18.15 % in Figure 4.

The secondary structures of hulless barley protein analysis of the data obtained from the FTIR and CD show that similar results, which are all largely β-sheet (44.60 % and 48.27 %, respectively), the rest being mainly in β-turn (35.67 % and 36.47 %, respectively), and followed by α-helix (19.73 % and 18.15 %, respectively), the difference was not significant.

DISCUSSIONS

Infrared signals of microorganisms are highly specific fingerprint-like patterns that can be used for probing the identity of microorganisms. The simplicity and versatility of FTIR makes it a versatile technique for rapid differentiation, classification, identification and large-scale screening at the subspecies level[10]. Because water absorbs strongly in the most important spectral region, around 1640 cm^{-1}, studies in aqueous solution are difficult unless deuterium oxide is used as a solvent[11]. In the spectrum of concanavalin the lower frequency beta-structure band has components at 1622, 1634, and 1639 cm^{-1}. In proteins where this band is not split, the band center varies from 1633 cm^{-1} in the spectrum of ribonuclease S to 1638 cm^{-1} in the spectrum of trypain[12]. Normally, polypeptides and proteins have some characteristic absorption bands in infrared; for example, amide I region is about 1700-1600 cm^{-1}, amide II region is about 1550-1530 cm^{-1}, and amide III region is about 1300-1260 cm^{-1}. According to the

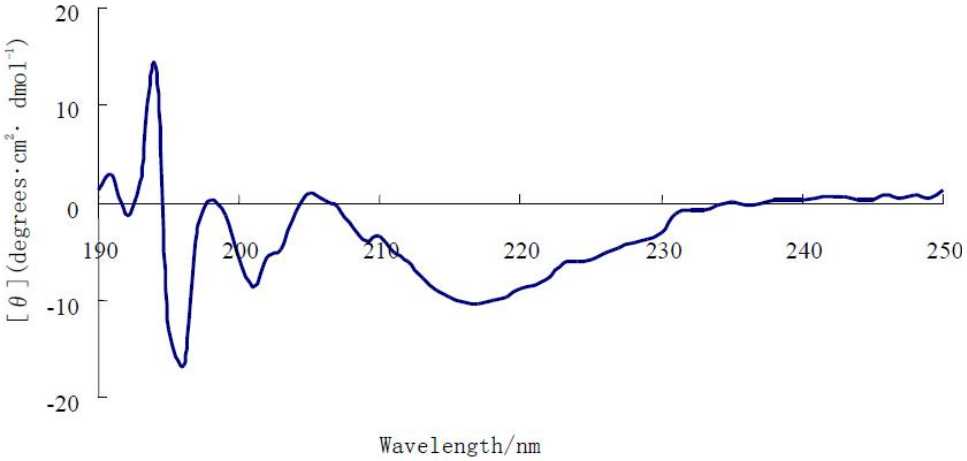

Figure 4 : CD spectra of hulless barley protein

descriptions of amide modes derived by Miyazawa et al.[13] and Krimm et al.[14], the amide I absorption contains contribution from the C=O stretching vibration of the amide group (about 80 %) with a minor contribution from the C-N stretching vibration, while the amide II absorption appears to be significantly less "pure", arising from N-H bending (60 %) and C-N (40 %) vibrations[5].

CD relies on the differential absorption of left and right circularly polarized radiation by chromophores which either possesses a number of chromophores which can give rise to CD signals. In the far UV region (180-240 nm), which corresponds to peptide bond absorption, the CD spectrum can be analyzed to give the content of regular secondary structural features such as α-helix, β-sheet, and β-turn [15]. The CD spectrum in the near UV region (320-260 nm) reflects the environments of the aromatic amino acid side chains and thus gives information about the tertiary structure of the protein[15]. There are a negative peak at near 216 nm and a positive absorption band in 185-200 nm, when it is the structure of β-sheets in samples. The characteristic absorption peak of β-turn exists in about 206 nm. In the far UV region (180-240 nm), there are a positive absorption peak at near 192 nm and two negative features acromion bands in 208 nm and 222 nm, when α-helixes consist in the samples. It can be sensitive to reflect solution protein conformation information in the far UV region, which it is analyzed using regular fitting algorithm to deal with the data of CD, and to work out the proportion of protein secondary structures in the solution.

Analysis of the secondary structures of proteins with low amounts of α-helix has in the past been difficult[6]. Indeed, it was easy to assume that the small amount of α-helix detected by CD was insignificant, especially with spectra that extended to only 200 nm[16]. With the recent advances in FTIR spectroscopy of proteins in aqueous solution, it has become possible to assign peaks in the amide I region to different forms of secondary structure. Several factors limit the accuracy with which FTIR and CD can determine protein secondary structure, such as sample state, approaches of analysis data, and the environment of determination. Both methods assume that elements of secondary structure contribute independently to the observed spectroscopic property.

CONCLUSIONS

The results showed that the methods of the FTIR and CD could effectively detect the secondary structure of hulless barley proteins, and the difference was not significant. Because the contents of *β*-sheet and *β*-turn were about 40 %, both more than α-helix, structural features of the hulless barley protein were spherical and relatively stable.

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