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Research Article

Structural Changes in Rice Bran Protein upon Different Extrusion Temperatures: A Raman Spectroscopy Study

Linyi Zhou,[1] Yong Yang,[1,2,3] Haibin Ren,[1] Yan Zhao,[1] Zhongjiang Wang,[1] Fei Wu,[1] and Zhigang Xiao[1,4]

[1]*College of Food Science, Northeast Agricultural University, Harbin, Heilongjiang 150030, China*
[2]*Key Laboratory of Processing Agricultural Products of Heilongjiang Province, College of Food and Bioengineering, Qiqihar University, Qiqihar, Heilongjiang 161006, China*
[3]*College of Food and Biological Engineering, Qiqihar University, Qiqihar, Heilongjiang 161006, China*
[4]*College of Grain Science and Technology, Shenyang Normal University, Shenyang, Liaoning 110034, China*

Correspondence should be addressed to Fei Wu; wufeiname@163.com and Zhigang Xiao; zhigangx@sina.com

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Raman spectroscopy is critically evaluated to establish the limits to which it may be used to detect changes in protein conformation upon extrusion. Rice bran protein (RBP) extruded with different temperatures (100, 120, 140, and 160°C, labeled as ERBP-) was considered. DSC showed that extrusion at 100°C increased T_D of RBP but decreased its ΔH, while, after extrusion treatment at 120°C, RBP completely denatured. A progressive increase in unordered structure and a general decrease in α-helix structure and β-sheet structure of extruded RBP were observed from Raman study. Meanwhile the content of unordered structure increased up to 140°C and then decreased at 160°C, while the trend of α-helix and β-sheet content was opposite, which was contributed to the composite effect of formation of some more protein aggregation and protein denaturation. Extrusion generally induced a significant decrease in Trp band near 760 cm^{-1} but an increase at 160°C. No significant difference was observed in Tyr doublet ratios between controlled RBP samples and extruded RBP below 160°C, whereas Tyr doublet ratios of extruded RBP decreased at 160°C. Intensity of the band assigned to CH_n bending decreased progressively and then increased as extrusion temperature increased, indicating changes in microenvironment and polarity.

1. Introduction

Rice bran, a major coproduct in the rice milling industry, usually contains about 11.3–14.9% protein, 34.0–62.0% carbohydrates (mainly starch), and 15.0–19.7% oil. In spite of being an excellent nutrient source, raw rice bran is not suitable for human consumption due to the rancidity problem caused mainly by lipases. When bran layers are removed from the endosperm during the milling process, the individual cells are disrupted and lipase enzymes come into contact with fat, causing hydrolysis of fat to free fatty acids (FFA) and glycerol [1]. However, stabilization, which is an enzyme inactivation process that extends the shelf life of rice bran, enables incorporation of rice bran back into our diet [2]. Extrusion stabilization is one of the most effect stabilization technologies. Most recent research on extrusion stabilization focuses on the quality and extraction rate of rice bran oil, but the study on structural and functional properties of extruded rice bran protein is rare. It has been suggested that, during extrusion, the combined effect of high temperature, high pressure, and shear forces in the extruder leads soy proteins to form laminated structures, which were easily accessible to enzymes [3]. Product characteristics of extrudes can vary considerably depending on the extrusion processing conditions such as barrel temperature, die geometry, extruder type, feed composition, feed moisture content, feed particle size, feed rate, screw configuration, and screw speed [4]. Moreover, extrusion temperature is one of the most important factors which may affect the quality of extruder. Qi and Onwulata [5] suggested certain functional properties of the extrudates

may be controlled by varying the extrusion temperature. Onwulata et al. [6] reported gel strength of extruded WPI at 35 or 50°C increased but almost was lost at 75 or 100°C. Thus, the effect of extrusion temperature on structure of rice bran protein was studied in this research.

Differential scanning calorimetry (DSC) has gained remarkable popularity in thermal studies of foods and their components following the development of instrumentation of sufficient sensitivity. DSC studies were often used to provide a better insight into the order-disorder transition processes of granular starch and other gelling polysaccharides. DSC also can be used to characterize mixtures of polymorphic forms of fats as well as to evaluate hydrogenation and various tempering regimes for their effectiveness in bringing about desired polymorphic changes. It has been employed to examine the physical state and properties of water in foodstuffs [7]. Above all, it is an excellent method for obtaining thermodynamic data on the unfolding of globular proteins and can provide unique information on the presence and characteristics of stable intermediates [8]. DSC is rapid, facile, and capable of supplying both thermodynamic (heat capacity, enthalpy, and entropy) and kinetic data (reaction rate and activation energy) on protein denaturation. With regard to proteins, denaturation curves result from thermal changes associated with the breakdown of those bonds involved in stabilizing protein structure (e.g., hydrogen bonds, hydrophobic interactions, and electrostatic interactions,) and, in some cases, an additional aggregation process [9].

Various techniques are currently available for obtaining the structural information of proteins, including X-ray diffraction, nuclear magnetic resonance (NMR) spectroscopy, and Fourier transform infrared spectra (FTIR). These methods have limitations. IR spectroscopic techniques could not be applied in solution to provide direct information about the structural changes. X-ray diffraction requires the preparation of perfect single crystal, which can be time-consuming or even impossible. NMR spectroscopy is not easily applied to proteins larger than a few hundred residues [10]. Moreover, studies on protein structure carried out in solution do not necessarily reflect what happens with proteins in the solid state [11]. Raman spectroscopy is a more suitable and direct technique that overcomes most of the above objections and can be used for solid samples and aqueous solutions due to weak background scattering from water, providing information on the peptide backbone structure, the environment of some side chains such as those of tyrosine and tryptophan (hydrophobic groups), and the local conformations of disulphide bonds and methionine residues [12]. In this respect, the usefulness of the Raman spectroscopy for the study of protein structural changes in food in situ during processing and storage has been shown, together with the possibilities of using protein structural changes, to predict protein functional properties and sensory attributes [12]. Raman spectroscopy was therefore used in the present research with the aim of studying the structural changes of rice bran protein (RBP) prepared under different extrusion temperatures. In general, the aim of this research was studying the protein denaturation and structural changes occurred in the rice bran protein subjected to the different extrusion temperatures using DSC and Raman spectroscopy.

2. Materials and Methods

2.1. Raw Materials. Deoiling rice bran was obtained from Orient Group (Harbin, China). All other chemicals were analytical grade products obtained commercially.

2.2. Preparation of Rice Bran Protein (RBP). Deoiled rice bran was ground to pass a 60-mesh screen and 10 g was dispersed in 100 mL distilled water. The protein was solubilized by adjusting the pH to 9.5 with 0.1 M NaOH and shaking at 300 rpm for 2.0 h at 50°C. The mainly nonprotein residue was removed by centrifugation at 3000 ×g for 20 min. The protein in the supernatant was precipitated by adjusting pH to 3.8 with 0.1 M HCl and centrifuged at 3000 ×g for 20 min. The precipitated protein was centrifuged and washed with distilled water two times, and the pH was brought up to 7.0 before freeze-drying. This protein was called RBP. The protein content of RBP was 89.59%.

2.3. Extrusion Treatment of Rice Bran. Deoiled rice bran was subjected to extrusion in a laboratory-scale twin-screw extruder (SPJ-40, Deai Co., Ltd, Shanxi, China) with three individual barrel sections, each with separate temperature control. The temperature of these three barrels was set at 50°C (feed section), 80°C, and 100~160°C, respectively. The rice bran blends were extruded at four different die-exit temperatures: 100, 120, 140, or 160°C. The diameter of the screw was 75 mm, and the length-to-diameter ratio was 28 : 1. The screw elements included kneading blocks and reverse screw elements. Screw speed was operated at 220 rpm. The die was designed with two circular holes at 5 mm diameter.

The moisture content of rice bran was adjusted to 20% specified for the extruder. Moisturized raw material was introduced to the extruder at a rate of 0.2 kg/min. The obtained extrudes of rice bran were ground to pass a 0.2 mm screen and then oven-dried at 50°C to reach a final moisture content of 5.1% (w/w). Ground extruded rice bran was stored in air-tight glass containers. Rice bran protein was prepared as the above-mentioned method and labeled as ERBP-100, ERBP-120, ERBP-140, or ERBP-160, respectively, corresponding to different extrusion temperatures.

2.4. Differential Scanning Calorimetry (DSC). DSC were recorded on a 2920 modulated DSC (TA Instrument, New Castle, DE) with heating rate of 5°C/min and temperature range of 25–120°C. The instrument was calibrated for temperature and enthalpy measurement with indium. Each hermetic aluminum pan was filled with 60 mg of 8% (w/w) rice bran protein dispersions in distilled water and sealed. An empty pan was used as reference. The enthalpy of denaturation (ΔH) and the temperature of denaturation (T_D) were calculated by using the DSC software after manually setting the start and the end points of the endothermic peak.

2.5. Raman Spectroscopic Analysis. The RBPs were dispersed in pH 7.0 phosphate buffers to make 100 mg/mL solutions

for Raman experimentation. Raman spectra were recorded on a Perkin Elmer Raman Station 400F Dispersive Raman Spectrometer equipped with a 785 nm diode laser which was used depending on the fluorescence contribution of the sample. The laser was focused on the samples which were placed on microscope slides. Each spectrum was obtained under the following conditions: 80 mW of laser power; 4 scans; 60 s exposure time; 2 cm^{-1} resolution; and the range of Raman spectra measured was 400–2000 cm^{-1}. Each sample was scanned at least three times, and the Raman spectra of each sample were plotted after calculating the mean. Errors in band position were less than ±3 cm^{-1}.

Spectral data from the scans of samples were smoothed, baseline-corrected, and normalized against the phenylalanine band at 1003 ± 1 cm^{-1} using the Grams 32 Software (Galactic Industries Corporation, Salem, NH, USA). The Phe band located near 1003 cm^{-1} was used as internal standard to normalize the spectra, as it has been reported to be insensitive to the microenvironment [12]. Assignment of the visible bands to vibrational modes of peptide backbone or amino acid side chains was carried out by comparing Raman spectra of model polypeptides or monographs of Raman spectra of proteins with those of in the references [13]. Quantitative estimation of secondary structure of RBP under specific conditions was performed using the Peakfit 4.12 software (Seasolve Software, Framingham, MA). Raman spectra (400–2000 cm^{-1}) were plotted as relative intensity (arbitrary units) against Raman shift in wavenumber (cm^{-1}). Raman spectra of each sample were collected in triplicate and the results were reported as the averages of these replicates.

2.6. Statistical Analysis. All experiments were performed in triplicate. Statistical analysis was performed using Statistical Analysis System (SAS 8.12, SAS Institute Inc., Cary, NC). Analysis of variance (one-way ANOVA) was employed taking relative intensity of each band as variables to determine the significance of different extrusion temperatures. The normal distribution of samples was checked using the Shapiro-Wilks test. The Kruskal-Wallis test was used to test samples that did not fit the normal distribution. Significant differences ($P < 0.05$) between means were identified using Duncan's multiple range test.

3. Results and Discussion

3.1. Thermal Characteristics of RBP Determined by DSC. Differential scanning calorimetry (DSC) can reveal structural and conformational changes of proteins. The denaturation temperatures (T_D, peak of the denaturation curve) and ΔH (enthalpy of the denaturation) both can be determined from the thermograms. Denaturation temperatures indicate protein thermostability, while ΔH is an indication of hydrophobic/hydrophilic interactions and compactness of the proteins.

RBP measured by DSC had a denaturation temperature of 79.9°C with endotherm of 1.70 J/g, which was consistent with pervious works. Pervious research reported that RBPI had denaturation temperature of 83.4°C with low endotherm (0.96 J/g of protein) [14]. Tang et al. [15] reported that denaturation temperatures of freeze-dried and spray-dried rice bran proteins were 84.1 and 84.6°C and enthalpies of them were 2.5 and 2.37 J/g, respectively.

Table 1: Differential scanning calorimetric (DSC) characteristics of rice bran protein (RBP) and extruded rice bran protein (ERBP) at different extrusion temperatures.

Sample	T_D (°C)	ΔH^{***} (J/g)
RBP*	79.9 ± 0.1[a**]	1.70 ± 0.10[b]
ERBP-100	82.3 ± 0.3[b]	1.54 ± 0.08[a]
ERBP-120	—	—
ERBP-140	—	—
ERBP-160	—	—

*RBP: rice bran protein; ERBP-100: extruded rice bran protein at 100°C temperature; ERBP-120: extruded rice bran protein at 120°C temperature; ERBP-140: extruded rice bran protein at 140°C temperature; ERBP-160: extruded rice bran protein at 160°C temperature.
**Different superscript letters in the same column indicate significant differences ($P < 0.05$).
***T_D means peak of the denaturation curve and ΔH means enthalpy of the denaturation.

It was observed from Table 1 that T_D of RBP increased from 79.9 to 82.3°C with an extrusion treatment at 100°C and ΔH was decreased from 1.70 to 1.54 J/g. Those observed results suggest that extrusion may result in a part denaturation of rice bran protein and partial exposure of hydrophobic cores initially buried in the interior; as a result the partially dissociated RBPs are refolded to form more stable aggregates with higher T_D, while, after extrusion treatment at 120°C, the endothermic peak of RBP disappeared, indicating complete denaturation of RBP.

3.2. Raman Spectroscopic Analysis. The Raman spectra of the controlled RBP and extrusion pretreated RBP at different temperatures are shown in Figure 1(a), respectively, and data for selected bands are shown in Table 2. Figure 1(b) showed the Raman band attributed to Trp and Tyr residues of RBP to give a clearly visible spectrum of these bands. The assignments of some major bands (Table 1) were made base on previous works [10]. The frequency and intensity changes in the Raman bands were mainly indicative of changes in the secondary structure and variations in local environments of RBP.

3.3. Amide Conformations Region. The conformations of RBP were mainly determined by the Raman characteristic bands of amide I band and amide III band. The Raman characteristic bands of amide I band were located as follows: α-helix, 1645–1660 cm^{-1}; β-sheet, 1665–1680 cm^{-1}; β-turn, 1680–1690 cm^{-1}; random coil, 1660–1670 cm^{-1} [10]. The Raman characteristic bands of amide III band were located as follows: α-helix, 1265–1300 cm^{-1}; β-sheet, 1230–1240 cm^{-1}; β-turn, 1305 cm^{-1}; random coil, 1240–1260 cm^{-1} [16]. The quantitative calculation of the secondary structures of the RBP Raman spectra was performed using the amide I band with Peakfit 4.12 software. Amide III bands were not used for quantifying because vibrational spectroscopy of proteins

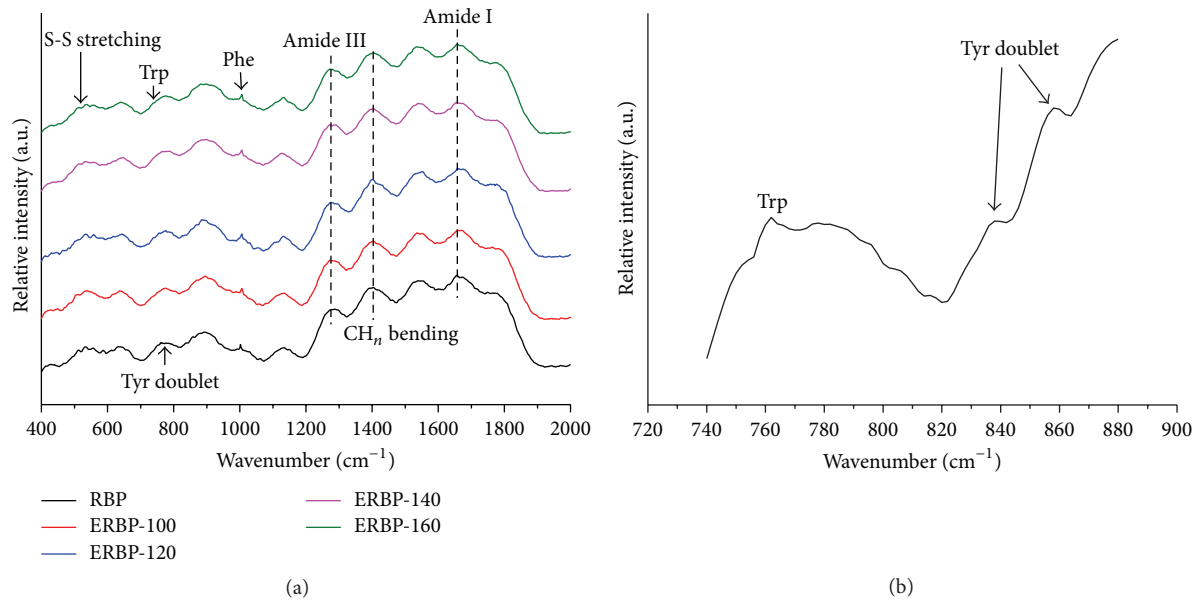

Figure 1: Raman spectrum of rice bran protein (RBP) and extruded rice bran protein (ERBP) at 100°C, 120°C, 140°C, and 160°C (a) and detailed spectrum of rice bran protein in 740–880 cm^{-1} region which include Trp band and Tyr doublet (b).

Table 2: Tentative assignment of some bands in the Raman spectrum of rice bran protein (RBP).

Frequency (cm^{-1})	Assignment
514	*νS-S *gauche-gauche-gauche* conformation
530	νS-S *gauche-gauche-trans* conformation
547	νS-S *trans-gauche-trans* conformation
620–640	Phenylalanine (Phe)**
644	Tyrosine (Tyr)
760	Tryptophan (Trp)
830	Tyr ν-ring
850	Tyr ν-ring
940	νC-C (α-helix)
1003	Phe ν-ring
1250	Amide III bands (β-sheet, random coil)
1273	Amide III bands (α-helix)
1309	Amide III bands (α-helix)
1321	Trp ν-ring
1340	δCH
1360	Trp ν-ring
1450	$\delta_{vs}CH_3$, δCH_2, δCH
1645–1690	Amide I bands

*ν, stretching vibrations; δ, bending vibrations; vs, very strong.
**Phe, phenylalanine; Tyr, tyrosine; Trp, tryptophan; α-helix, β-sheet, β-turn, and unordered structure are secondary structure elements of protein.

produces a complex pattern of bands in this range (located in the 1200–1300 cm^{-1}) [16].

Raman bands corresponding to amide I and amide III can be used to characterize protein backbone conformation. Amide I band region is mainly due to the stretching vibrations of the carbonyl groups and is representative of the secondary structure of the proteins [17]. These absorption bands allow the assignment of secondary structure of proteins. The strong Raman band centered at 1665–1675 cm^{-1} has been assigned unambiguously to the amide I vibrational mode, which involves mainly C=O stretching vibrations and partly N-H in-plane bending of peptide groups [12]. The strongest Raman band at 1665–1670 cm^{-1} demonstrated the predominance of β-sheet in RBP. The spectral profile of the amide I band is used for quantifying the secondary structure of proteins [18]. Table 3 lists the secondary structure percentages from samples analyzed. Results showed that nontreated RBP contained 17.43% α-helix, 41.14% β-sheet, 19.67% turns, and 19.76% unordered structures (Table 3). Adebiyi et al. [19] reported the secondary structure of rice bran globulin may be summarized to be an unordered, random coil, and antiparallel chain of intramolecular β-sheet structure, while rice bran albumin may be composed of α-helix conformation and ordered structure with intermolecular β-sheet. The high content of β-sheet structure of RBP in this study may be attributed to the high percentage of rice bran albumin and globulin. Ma et al. [20] suggested the overall secondary structures of RBPF were very similar to those of plant proteins having low α-helix, large contents of β-sheet.

The estimated secondary structure results from Table 3 showed a progressive increase in unordered structure and a general decrease in α-helix structure and β-sheet structure of extruded RBP in comparison with controlled RBP sample. Qi and Onwulata [5] reported that extrusion at or above 75°C leads to a uniform densely packed polymeric product with no secondary structural elements (mostly α-helix) remaining. Qi and Onwulata [21] reported that protein denaturation may decrease its β-sheet content.

Table 3: Percentages of protein secondary structure: α-helix, β-sheet, β-turn and unordered structure of rice bran protein (RBP) and extruded rice bran protein (ERBP) at different extrusion temperature.

Sample	Percentage of secondary structure elements (%)			
	α-helix structure	β-sheet structure	β-turn structure	Unordered structure***
RBP*	17.43 ± 0.01c**	41.14 ± 0.04e	19.67 ± 0.02c	19.76 ± 0.01a
ERBP-100	17.83 ± 0.02d	38.00 ± 0.03d	19.67 ± 0.04c	23.50 ± 0.02b
ERBP-120	17.08 ± 0.03b	34.51 ± 0.02b	19.33 ± 0.03a	29.08 ± 0.01d
ERBP-140	17.01 ± 0.02a	31.83 ± 0.04a	19.32 ± 0.00a	31.83 ± 0.03e
ERBP-160	18.31 ± 0.01e	36.31 ± 0.02c	19.59 ± 0.01b	25.78 ± 0.03c

*RBP: rice bran protein; ERBP-100: extruded rice bran protein at 100°C temperature; ERBP-120: extruded rice bran protein at 120°C temperature; ERBP-140: extruded rice bran protein at 140°C temperature; ERBP-160: extruded rice bran protein at 160°C temperature.
**Different superscript letters in the same column indicate significant differences ($P < 0.05$).
***α-helix, β-sheet, β-turn, and unordered structure are secondary structure elements of protein.

It was observed that the content of unordered structure increased up to 140°C and then decreased at 160°C, while the trend of α-helix and β-sheet content was opposite. Heat and shear alter the conformation of proteins through partial denaturation of the protein molecules, exposing groups that are normally concealed in the folded native protein, resulting in an increase of unordered structure [22]. Those observed Raman results were consistent with DSC study which suggest that extrusion may result in a denaturation of rice bran protein and partial exposure of hydrophobic cores initially buried in the interior. Proteins texturized at the higher temperature showed an increase in surface hydrophobicity and unfolding of protein structure [22], which may result in a progressively increase in unordered structure. Qi and Onwulata [5] indicated that extrusion results in a loss of secondary structure of around 15%, total loss of globular structure at 78°C, and conversion to a random coil at 100°C.

The decreased unordered structure and increased β-sheet structure at 160°C extrusion temperature may be related to the formation of more or new protein aggregation. Chen et al. [23] reported that increasing moisture content from 28% to 60% or decreasing cooking temperature from 160 to 140°C all could reduce the degree of aggregation in extruded soybean protein. Choi and Ma pointed that protein aggregation may increase its antiparallel β-sheet conformation. Lee et al. [24] also reported that β-sheets played a role in the aggregate and network formation. As previously reported by Simmons et al. [25], heating at different temperatures and in the presence of shear resulted in two types of aggregates. At lower temperatures, small and weakly bonded aggregates were formed, due to weak van der Waals bonding, while rigid and dense aggregates were observed after heating at higher temperatures. In the present study, it could be assumed that the protein aggregates formed in ERBP-160 are rigid-textured aggregates, which may result in a decrease in unordered structure and an increase in β-sheet structure. However, the correctness of this speculation still needs to be further confirmed.

3.4. Local Environments of Proteins

3.4.1. Tryptophan Residues. Tryptophan (Trp) residues show several characteristic Raman bands, some of which are useful to monitor the polarity of the microenvironment, or involvement in hydrogen bonding. Li-Chan [26] reported that tryptophan residues from a buried, hydrophobic microenvironment become exposed to the polar aqueous solvent; there may be a decrease in the intensity of a band near 760 cm^{-1} region. Pervious works often reported a decrease in intensity of Trp band of denatured proteins due to more exposed Trp residues caused by destruction and unfolding of protein structure [27].

However, in this study, extrusion induced a significant decrease in the stretching vibration of the tryptophan residues ring, which indicated that tryptophan residues tended to an exposed, hydrophilic microenvironment. Taylor et al. [28] reported that extrusion process frequently results in realignment of disulfide bonds and breakage of intramolecular bonds, while disulfide bonds stabilize the tertiary structure of protein and may limit protein unfolding during extrusion. The increase in normalized intensity of Trp band of RBP extruded at 160°C may contribute to the formation of aggregation or new more ordered structure [29]. These observed results are consistent with changes found in secondary structure analysis and DSC analysis, while the decreased ΔH of ERBP in DSC confirmed those observed results.

3.4.2. Tyrosine Doublet Ratio. The tyrosyl (Tyr) doublet ratio (I_{850}/I_{830}) can be useful in monitoring the microenvironment around tyrosyl residues. In fact, the I_{850}/I_{830} ratio achieved its minimum value of about 0.3 when tyrosine residues were buried and the phenolic OH group acted as a strong hydrogen bond donor to an electronegative acceptor, such as carboxyl oxygen [30]. When tyrosines were exposed at the surface of the proteins, the phenolic OH acted as both a donor and an acceptor of moderate hydrogen bonds, and the I_{850}/I_{830} was approximately 1.25 [30]. The ratio ranged from 0.95 to 1.01 in this experiment, which suggested that the tyrosine residues of ERBP were exposed to the aqueous or polar microenvironment or act as simultaneous acceptor and donor of moderate to weak hydrogen bonds. From Table 4, there was no significant difference in Tyr doublet ratios between controlled RBP and extruded RBP below 160°C, which indicated that natural, exposed Tyr residues to the aqueous or polar microenvironment had remained

Table 4: Normalized intensities of the tryptophan stretching band (759 cm^{-1}), tyrosyl doublet stretching ring (850/830 cm^{-1}), CH bending vibration band (1450 cm^{-1}) of rice bran protein (RBP), and extruded rice bran protein (ERBP) at different extrusion temperature.

Sample*	Trp band (I_{760}/I_{1003} cm^{-1}**)	Tyr doublet (I_{850}/I_{830} cm^{-1})	CH band (I_{1450}/I_{1003} cm^{-1})
RBP	$1.03 \pm 0.01^{d***}$	1.01 ± 0.01^{b}	1.11 ± 0.01^{e}
ERBP-100	1.00 ± 0.01^{c}	1.00 ± 0.00^{b}	0.95 ± 0.00^{c}
ERBP-120	0.97 ± 0.00^{b}	1.01 ± 0.01^{b}	0.92 ± 0.01^{b}
ERBP-140	0.94 ± 0.01^{a}	1.00 ± 0.00^{b}	0.88 ± 0.00^{a}
ERBP-160	0.98 ± 0.01^{b}	0.95 ± 0.01^{a}	0.98 ± 0.01^{d}

*RBP: rice bran protein; ERBP-100: extruded rice bran protein at 100°C temperature; ERBP-120: extruded rice bran protein at 120°C temperature; ERBP-140: extruded rice bran protein at 140°C temperature; ERBP-160: extruded rice bran protein at 160°C temperature.
**Normalized intensities are the ratio of relative intensity of each Raman band and intensity of phenylalanine band at 1003 cm^{-1}.
***Different superscript letters in the same column indicate significant differences ($P < 0.05$).

during extrusion process. Extruded RBP at 160°C decreased its tyrosine doublet $I_{850/830}$ ratios, which could be contributed to formation of more protein aggregation. Ikeda and Li-Chan [31] reported that protein denaturation makes a protein molecule to partially unfold, followed by aggregation; in this case, a more compact protein was formed and the initially exposed tyrosine residues were buried into the intermolecular interface. Herrero et al. [16] also reported that heated SPI reveal either increasing buriedness or involvement of Tyr residues as strong hydrogen bond donors when comparing refrigerated and heated samples.

3.5. Aliphatic C-H Bending Vibration. The band assigned to the CH_2 and CH_3 bending vibrations is observed near 1450 cm^{-1}. In the 2800–3050 cm^{-1} region of the Raman spectrum, aliphatic amino acids, peptides, and proteins exhibit C-H stretching vibrations. Only the CH_2 and CH_3 bending vibrations (1450 cm^{-1}) band was studied, because no changes were observed in the intensity of the 2930 cm^{-1} band which increased with increasing polarity of the environment aliphatic hydrophobic side chains of proteins.

A decrease in the intensity of these bands indicates exposure of aliphatic residues, while an increase indicates buried residues [12]. However, some researchers have argued that an increase in C-H bending intensity suggests exposure of hydrophobic groups to a more polar environment [32]. The indicators of changes in C-H bending vibration are controversial, but it is commonly thought that changes in C-H bending vibration relate to changes in tertiary structure of proteins. An overall tendency of intensity decreasing of 1450 cm^{-1} band was observed from Table 4 in comparison of controlled samples; it can be ascribed to the fact that tryptophan residues tend to expose to a hydrophilic microenvironment. Moreover, the intensity of 1450 cm^{-1} band decreased progressively and then increased as extrusion temperature increased, indicating changes in microenvironment and polarity. Pervious works reported these results can be attributed to decreased interior hydrophobic interactions and increased exposure of hydrophobic groups to a more polar environment due to unfolding of protein during denaturation [27]. The decreased intensity of this band may be also attributed to denaturation of extruded rice bran protein as determined in DSC study.

4. Conclusions

Raman spectroscopy was used to determine structural changes in RBP during extrusion process. Raman study observed extrusion progressively increased unordered structure but general decrease in α-helix structure and β-sheet structure of RBP. In conclusion, thermal denaturation in extrusion process generally increased the content of unordered structure and exposed more hydrophobic Trp residues, while aggregation formation when extruding RBP at 160°C increased α-helix and β-sheet content and buried more hydrophobic Trp and Tyr residues. Intensity of the band assigned to CH_n bending decreased progressively and then increased as extrusion temperature increased, indicating changes in microenvironment and polarity. Though mechanism of denaturation and aggregation process of RBP remains to be clearly defined, Raman spectroscopy provides a feasible tool to study the structural changes of RBP prepared under different extrusion temperatures. The greater exposure of aromatic hydrophobic residues could improve the emulsifying and foaming properties of extruded RBP (especially at 140°C). Thus, the RBP extruded at different temperatures can be advantageously applied for various product applications.

Besides, frequently used spectra such as CD and NMR spectrum could not be applied in detecting RBP's structure for its low solubility, while FT-IR spectrum could hardly study the structure of protein in aqueous solution. Raman spectroscopy would be the most convenient analytical method that could be used for proteins in solution with low solubility and provide a direct, nondestructive, and faster determination of the structure of proteins and give a new perspective to elucidation of protein structure.

Conflict of Interests

All of the authors have no conflict of interests.

Authors' Contribution

Dr. Linyi Zhou and Dr. Yong Yang contributed equally to this research.

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