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Infrared and Raman Spectroscopic Characterization of Structural Changes in Albumin, Globulin, Glutelin, and Prolamin during Rice Aging

Yubao Guo,[†,‡] Weirong Cai,[‡] Kang Tu,[*,†] Sicong Tu,[§] Shunmin Wang,[‡] Xiuling Zhu,[‡] and Wei Zhang[†]

[†]College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, P. R. China

[‡]School of Biological and Chemical Engineering, Anhui Polytechnic University, Wuhu, Anhui 241000, P. R. China

[§]Faculty of Medicine, University of New South Wales, Sydney, Australia

Supporting Information

ABSTRACT: Structural changes in albumin, globulin, glutelin, and prolamin from fresh and aged rice were characterized in this study. Infrared and Raman spectroscopies revealed changes in interactions between protein and starch, and the occurrence of structural changes involving secondary and tertiary structures of protein induced by rice aging. The α-helical structure was reduced, and aliphatic amino-acid side chains became more buried in albumin after rice aging. Oxidation of the sulfhydryl group in globulin was evident. The unordered coil in glutelin decreased, and a characteristic frequency of the free sulfhydryl group appeared. The antiparallel β-sheet in prolamin increased, the conformation of disulfide bonds changed, and tyrosine residues became exposed to a polar environment. The association between globulin and starch strengthened, whereas that between glutelin and starch diminished. These differences in structure and interactions with starch might be responsible for the dissimilar pasting properties between fresh and aged rice.

KEYWORDS: *Raman spectroscopy, infrared spectroscopy, storage protein, rice aging, structural characterization*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most widely consumed cereal foods for about half of the world's population.[1] To sustain consumer demand throughout the year, harvested rice is subjected to varying periods of storage. Aging of rice is inevitable during storage.[2,3] Aging affects the processing, cooking, and eating quality of rice and has been widely studied to elucidate underlying mechanisms.[4−9] Nevertheless, the effects of aging process on rice properties are complicated, and the mechanisms of rice aging are still not fully understood.[10]

Attempts to explain the changes in functionality associated with rice aging have focused on the properties of the primary components in rice, such as starches, proteins, and lipids, and the interactions among them during storage.[10] Changing properties of the proteins by adding ascorbic acid, dithiothretiol, or sodium sulfite to flour from aged rice was found to affect the physicochemical properties of aged rice, showing the close relationship between rice proteins and the physicochemical properties of aged rice.[3,5,6,8,11−13] It has been shown that changes in rice proteins, rather than starches, are primarily responsible for rheological changes related to the aging of rice flour.[11,14−16] However, the content of protein in rice was not found to change during storage.[12] Therefore, research should be focused on the changes in protein structure and in interactions between protein and starch during rice storage, rather than on protein content.

Infrared and Raman spectroscopies, considered as two complementary vibrational spectroscopy techniques, have been applied to characterize the secondary and tertiary structural changes of proteins in meat induced by heating or salt,[17] as well as interactions between protein and lipid and among different proteins.[18−20] Structure characterizations of cereal proteins by infrared or Raman spectroscopy are limited.[21−24] To our knowledge, there exist no reports concerning infrared and Raman spectroscopic characterization of the structural changes in rice proteins and interactions between protein and starch, induced by rice aging during storage. The aim of the present study was to investigate structural changes in rice proteins and their interactions with starch during rice aging, which will facilitate an enhanced understanding of the rice aging mechanism.

MATERIALS AND METHODS

Materials and Rice Storage. Newly milled nonwaxy japonica rice, *Oryza sativa* L. cultivar Wuyujing 3, grown in Xuyu County, Jiangsu Province, China, was used in this study. The milled rice was divided into two portions, placed in airtight glass bottles, and stored at 4 °C in a refrigerator (considered as fresh rice) and at 45 °C in a thermostatically controlled incubator (considered as aged rice) for 6 months.[11] After storage, both bottles stored at 4 and 45 °C were removed and held at an ambient temperature of 15 °C for 48 h to balance the sample temperature. Moisture and total protein content were determined following the description of Chrastil and AACC Method 46-11A.[12,25] The moisture contents of fresh and aged rice were 13.29% and 13.20%, respectively.

Sample Preparation for Rice Protein Extraction. Samples (20 g each) of fresh or aged rice were ground to rice flour using a cyclone

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sample mill (Tester Corporation, Tianjin, China) for 20 s each time through a 0.45-mm sieve screen immediately prior to use.

Rice Protein Extraction. Rice proteins, including albumin, globulin, glutelin, and prolamin, were extracted individually from the rice flour, following the Osborne procedure described by Likitwattanasade and Hongsprabhas.[8] Prior to deproteinization, defatting was carried out to avoid the disturbance of the rice lipid. The residues of rice flour after deproteinization were dried in a freeze dryer for viscosity analysis through a rapid visco analyzer (RVA). Afterward, the lyophilized powder of individual protein, recovered from supernatant liquid, was stored at −18 °C before use for infrared and Raman spectroscopic analysis.[8] The determined contents of proteins in the lyophilized powders were 77.94% and 79.24% for albumin, 89.65% and 87.36% for globulin, 94.36% and 97.52% for glutelin, and 93.54% and 93.10% for prolamin in the fresh and aged samples, respectively.

Viscosity Analysis. A rapid visco analyzer (RVA, model Super 3, Newport Scientific Inc., Warriewood, Australia) was used to obtain pasting profiles utilizing Thermocline software following the AACC Method 61-02.[26] Each sample was run at least three times in the RVA, and means of three replications are presented. The selected parameters include peak viscosity, trough, and final viscosity.

Infrared Spectral Analysis. Infrared spectra of the rice storage proteins were recorded with a Fourier transform infrared spectrometer (FT-IR 200, Thermo Nicolet Corp., Madison, WI, USA) with Omnic 8.0 software in the range 4000−400 cm^{-1} using the KBr-disk method. The samples were pressed into KBr pellets with a sample/KBr ratio of 1:100. Sixty-four scans were accumulated to produce a spectrum for each of three pellets from the same sample, resulting in 192 scans per protein.

Raman Spectral Analysis. Samples were excited at 514.5 nm by an argon-ion laser at room temperature (25 °C), using a laser confocal microscopy Raman spectrophotometer (HR 800, Horiba Jobin-Yvon, Villeneuve d'Ascq, France) equipped with a 50× lens. The laser power was set at 20 mW. The laser spot diameter reaching the sample was about 1 μm. Before measurement, the laser wavelength was calibrated with monocrystalline silicon at 520.7 nm. For measurement, rice protein samples were placed on microscope slides. The laser was then focused on the sample. The Raman spectra of at least three different spots were recorded in the range of 400−4000 cm^{-1}. Each spectrum was collected under the following conditions: 60-s exposure time, 2 cm^{-1} resolution, sampling speed of 120 cm^{-1}/min with data recorded every 1 cm^{-1}. The spectral data were baseline-corrected, smoothed by a nine-point Golay−Savitzky procedure using Omnic 8.0, and normalized against the phenylalanine band at 1005 ± 2 cm^{-1}.[27]

Statistical Analysis. Statistical analysis of the data was carried out using SAS Software, version 8.0 (SAS Institute Inc., Cary, NC, USA). The data were analyzed by analysis of variance (ANOVA) using Duncan's multiple range tests at the significant level $p < 0.05$, and the mean values of three replicates are reported.

RESULTS AND DISCUSSION

Difference in Pasting Properties of Fresh and Aged Rice Flour Induced by Deproteinization. Pasting profiles of the defatted rice flour from fresh and aged rice and their counterparts of deproteinization fractions are shown in Figure 1. Before deproteinization, considerably different RVA profiles were observed between fresh and aged rice residues (Figure 1A,B). In particular, the aged rice had a higher trough and final viscosity, as well as a lower peak viscosity, compared to fresh rice, which are the typical characteristics of rice aging.[28] This finding shows that aging did occur in rice stored at 45 °C for 6 months. After deproteinization, the RVA profile of the aged rice residue (Figure 1D) was similar to that of the fresh rice residue (Figure 1C), as only a slightly higher peak viscosity and a slightly lower final viscosity were found in the RVA profile of the aged rice residue. The clear difference in the RVA profiles of fresh and aged rice residues before deproteinization almost disappeared after deproteinization, indicating the dominant

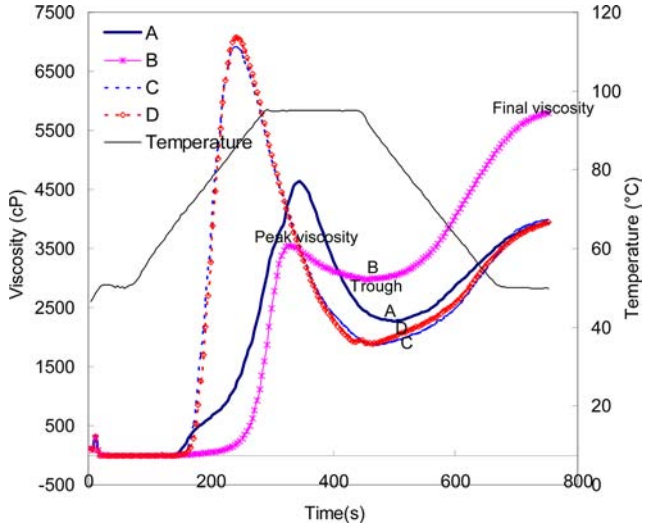

Figure 1. RVA pasting profiles of rice flour fractions: (A) defatted fresh rice flour, (B) defatted aged rice flour, (C) defatted and deproteinized fresh rice flour, and (D) defatted and deproteinized aged rice flour.

roles of rice proteins in the pasting properties of aged rice. This finding supports the hypothesis proposed by Teo et al., who asserted that modification of the protein component, rather than starch, is primarily responsible for rheological changes associated with the aging of rice flour.[11] The total protein content during rice aging did not change significantly (7.85% and 7.74% on a dry basis in fresh and aged rice, respectively), which is consistent with the results of Chrastil.[12] This initiates research focused on the changes in protein structures and interactions among proteins and starch.

Infrared Characterization of Structural Change in Rice Proteins Induced by Aging during Storage. In general, Raman spectroscopy is proficient at detecting symmetric vibrations of nonpolar groups, whereas infrared spectroscopy is more suited for determining asymmetric vibrations of polar groups. Infrared spectroscopy measures transitions between molecular vibrational energy levels as a result of the absorption of mid-infrared radiation. This interaction between light and matter is a resonance condition involving the electric-dipole-mediated transition between vibrational energy levels.[29]

Infrared Characterization of Structural Changes in Albumin Induced by Rice Aging. Infrared spectra of the albumins from fresh and aged rice in the 400−4000 cm^{-1} region are shown in Figure 2a. Assignments of the infrared bands were carried out according to previous literature reports.[29−31] No obvious differences relevant to absorbance frequency in albumins were found between fresh and aged rice. Regardless of the age of the rice, the amide I and II bands of the albumins were centered at 1654 and 1543 cm^{-1}, indicating a predominance of the α-helix. The band near 1153 cm^{-1} together with that at 1335 cm^{-1} suggest the existence of sulfone. The band centered at 1027 cm^{-1} and those at 1079 and 1153 cm^{-1} indicate the stretching vibration of C—O in saccharide, and the bands near 936, 864, and 759 cm^{-1} suggest skeletal vibrations of saccharide rings. Together, these results indicate that either the albumin is glycoprotein or it strongly interacts with starch. The decreased absorbance intensities in 1153, 1079, 1027, 936, 864, and 759 cm^{-1} show that rice aging reduced the association between starch and albumin (Table 1).

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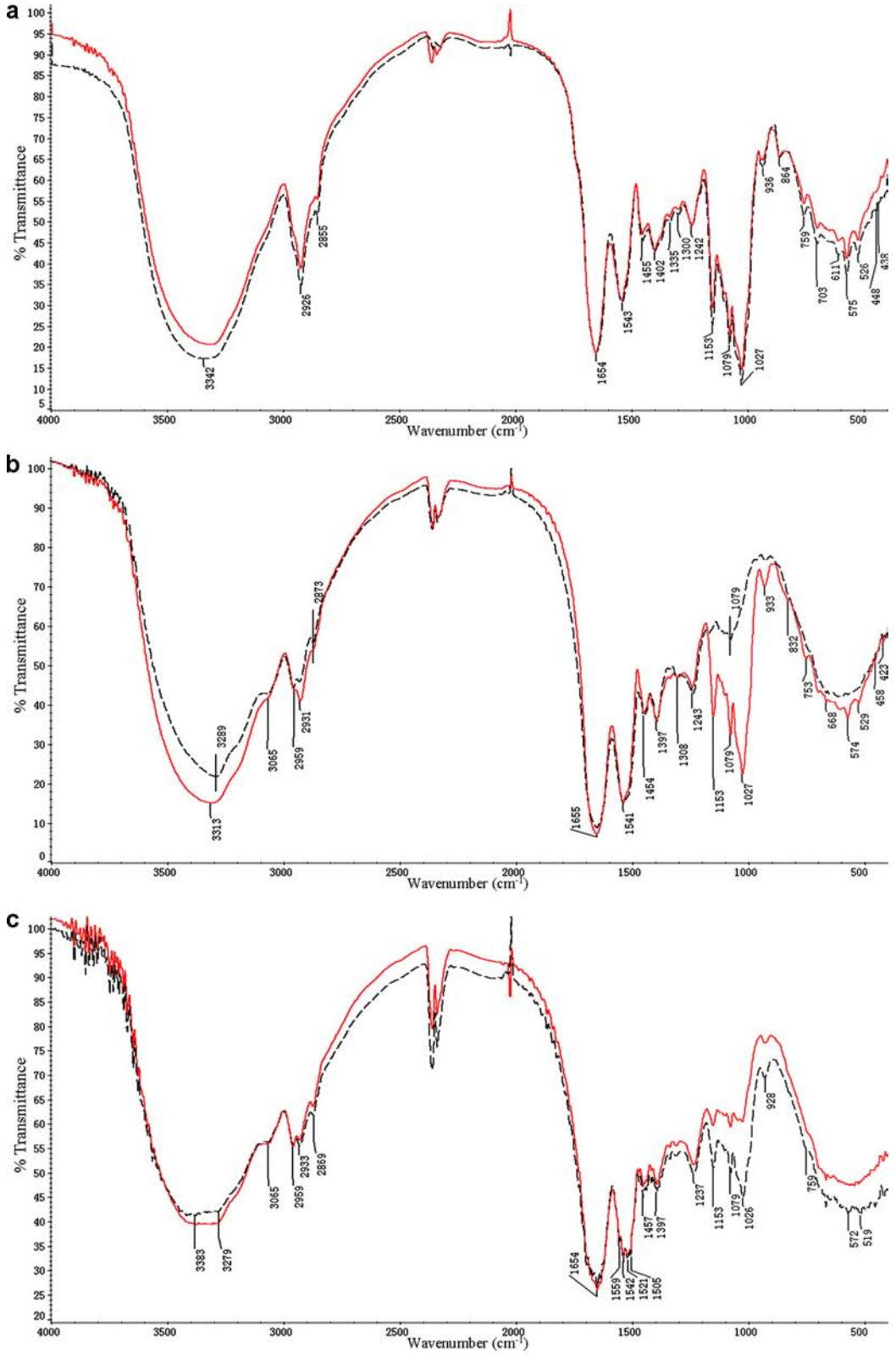

Figure 2. FT-IR spectra of (a) albumins, (b) globulins, and (c) glutelins extracted from fresh rice (dashed lines) and aged rice (solid lines).

Infrared Characterization of Structural Changes in Globulin Induced by Rice Aging. Infrared spectra of the globulins from fresh and aged rice in the 400−4000 cm^{-1} region are shown in Figure 2b. Globulin in aged rice showed increased absorbance intensities at the bands near 1153, 1079, and 1027 cm^{-1} (Table 1). The bands near 933 and 753 cm^{-1} also increased to varying degrees. The frequency of the maximal peak in the range of 3200−3600 cm^{-1} shifted from 3289 to 3313 cm^{-1} as a result of rice aging, indicating that contribution of the hydroxyl group to the maximal peak is greater than that of the amino group. This suggests that globulin has more intense interactions with starch in aged rice than in fresh rice.

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Table 1. IR Frequencies and Tentative Assignments of Proteins from Fresh and Aged Rice

	band position (cm^{-1})			normalized intensity[a] (A/A_{1654})	
sample	fresh rice protein	aged rice protein	assignment	fresh rice protein	aged rice protein
albumin	1654	1654	amide I	1.000	1.000
	1543	1543	amide II	0.706 a	0.692 a
	1335	1335	S═O	0.420 a	0.387 b
	1153	1153	S═O, C—O	0.817 a	0.716 b
	1079	1079	C—O	0.944 a	0.873 b
	1027	1027	C—O	1.252 a	1.144 b
	936	936	C—C	0.279 a	0.246 b
	864	864	C—C	0.256 a	0.233 b
	759	759	C—C	0.399 a	0.344 b
globulin	3289	3313	N—H, O—H	0.620 b	0.715 a
	1153	1153	S═O, C—O	0.212 b	0.379 a
	1079	1079	C—O	0.235 b	0.429 a
	1027	1027	C—O	0.176 b	0.565 a
	933	933	C—C	0.111 b	0.140 a
	832	832	C—C	0.161 a	0.159 a
	753	753	C—C	0.244 b	0.252 a
glutelin	1153	1153	S═O, C—O	0.561 a	0.389 b
	1079	1079	C—O	0.579 a	0.392 b
	1026	1027	C—O	0.657 a	0.380 b
	928	928	C—C	0.303 a	0.210 b

[a]Different letters (a, b) in the same row indicate significant differences ($p < 0.05$).

Infrared Characterization of Structural Changes in Glutelin Induced by Rice Aging. The infrared spectra of the glutelins from fresh and aged rice in the 400−4000 cm^{-1} region are different (Figure 2c). The frequencies corresponding to different intensities between glutelins in fresh and aged rice were similar to those of globulin. However, the pattern of absorbance intensity changes was opposite; that is, the intensities near 1153, 1079, and 1026 cm^{-1} decreased, and the amplitude of the change diminished compared with those of globulin. Coupled with the decreased intensity near 928 cm^{-1}, this indicates reduced interactions between glutelin and starch. Chrastil reported that the adsorption equilibrium constant between rice glutelin (orizynin) and starch decreased upon prolonged rice storage.[12] This is consistent with the current findings.

Infrared Characterization of Structural Changes in Prolamin Induced by Rice Aging. The infrared spectra of prolamins from fresh and aged rice are similar in the 400−4000 cm^{-1} region (see Figure 1S in the Supporting Information), and no information related to the interaction between prolamin and starch is involved. A possible explanation for this finding is that the interactions between prolamin and starch are minimal, which is supported by the fact that the RVA profiles of the reconstituted flour (prolamin added back to the extracted residue of rice flour) were almost identical to those of the original native flour,[32] and did not change significantly during rice aging.

Raman Characterization of Structural Changes in Rice Proteins Induced by Aging during Storage. Raman spectroscopy is a two-photon inelastic light-scattering event. The incident photon is of much greater energy than the vibrational quantum energy and loses part of its energy to the molecular vibration, with the remaining energy scattered as a photon with reduced frequency. In the case of Raman spectroscopy, the interaction between light and matter is an off-resonance condition involving the Raman polarizability of the molecule.[29]

Raman Characterization of Structural Changes in Albumin Induced by Rice Aging. Raman spectra of the albumins from fresh and aged rice in the 400−1700 cm^{-1} region are shown in Figure 3.

The amide I band of albumin in fresh rice was centered at 1662 cm^{-1}, whereas in aged rice, it was centered at 1660 cm^{-1}, indicating that the α-helix as the principal secondary structure increased after rice aging. The amide III band near 1256/1259 cm^{-1} supports the α-helix as the principal secondary structure.[33]

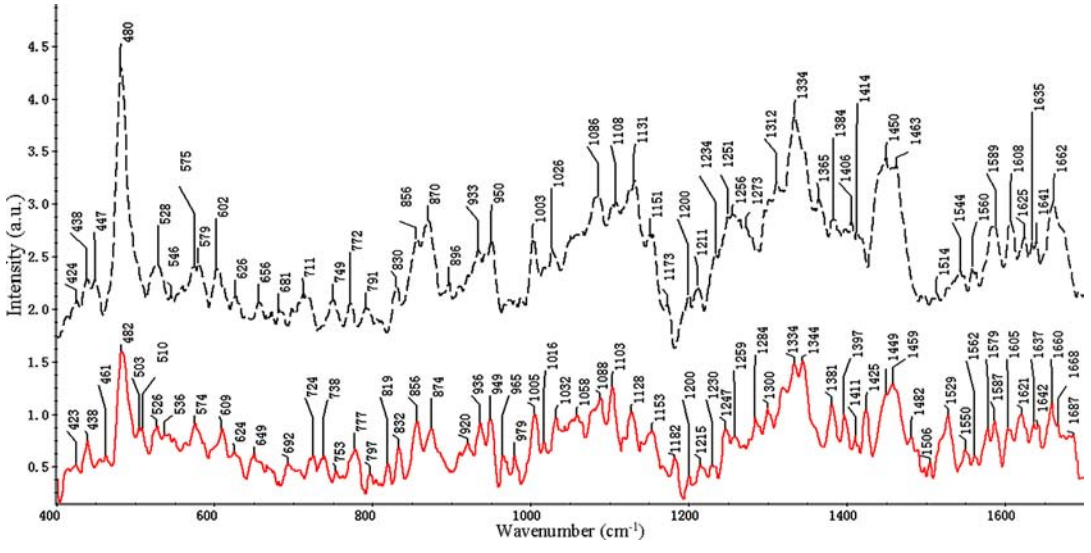

Figure 3. Raman spectra of albumins extracted from fresh rice (dashed line) and aged rice (solid line) in the 400−1700 cm^{-1} region.

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The band near 510 cm^{-1} in the Raman spectra of the proteins has been assigned to disulfide bonds in gauche−gauche−gauche conformation.[34] The bands near 525 and 545 cm^{-1} were attributed to disulfide bonds in the gauche−gauche−trans and trans−gauche−trans conformations, respectively,[34] although tryptophan residues might also contribute to the band at 540 cm^{-1}.[35] A decrease in the intensity of the albumin band around 528/526 cm^{-1} coupled with an increase in the intensity near 510 cm^{-1} was observed as a result of rice aging (Table 2). This shows that the disulfide-bond content in a gauche−gauche−trans extended conformation decreased, whereas that in a gauche−gauche−gauche conformation increased.

The bands near 750 and 1334 cm^{-1} provide information about the microenvironment of the tryptophan residues. Decreased intensity at 1334 cm^{-1} indicates more exposed tryptophan residues.[36] The intensity ratio of the tyrosine ring vibrations at 850 and 830 cm^{-1} represents "buried" and "exposed" tyrosine residues. The tyrosine intensity ratio decreased from 1.46 to 1.29 due to rice aging, but still remained greater than 1, indicating that the tyrosine residues became less exposed and became involved in hydrogen-bond donation to a greater extent.[36]

Raman spectra in the frequency range of 2800−3100 cm^{-1} are assigned to C—H stretching modes, which are attributed to the vibrations of CH, CH_2, and CH_3 groups in the side chains of amino-acid residues and are sensitive to the ionization state and residue microenvironment.[27] After rice aging, the intensity of the albumin C—H stretching bands decreased, suggesting that the aliphatic side chains were more buried and the molecular polarity of albumin increased.[37,38]

Another pronounced difference in albumins from fresh and aged rice is the intensity change in the bands at 933/936 and 480/482 cm^{-1}. Although the characteristic frequency ranges for peptide backbone (890−1060 cm^{-1}) and starch (940 cm^{-1}) overlap to some extent,[33] the band near 480 cm^{-1} is unique to starch.[22,31] As a result of rice aging, a decrease in the intensity at 480 cm^{-1} in the albumin spectrum was clearly seen, indicating that the association between albumin and starch was reduced.

Raman Characterization of Structural Changes in Globulin Induced by Rice Aging. Raman spectra of the globulins from fresh and aged rice in the 400−1700 cm^{-1} region are shown in Figure 4.

Significant changes were observed in the globulin spectra of fresh and aged rice. The amide I and III bands of fresh rice globulin appeared near 1656 and 1271 cm^{-1}, which clearly shows the predominance of the α-helical structure.[21] The amide I band of globulin from aged rice was centered at 1662 cm^{-1} and appeared with a high-intensity band near 1622 cm^{-1}, which represent unordered structure and β-sheet, respectively.[31,33,34] The C—C stretching band shifted from 943 to 955 cm^{-1}, and its intensity increased considerably, which suggests decreased α-helical structure in globulin of aged rice. In addition, a strong peak near 1438 cm^{-1} was presented, which was attributed to C—H bending motions.[39]

A change in disulfide conformation upon a shift in the dimer−monomer equilibrium of acid phosphatase has been reported,[40] indicating that the disulfide bridge peak could shift from 550 and 525 cm^{-1} in the dimer to 500 cm^{-1} in the monomer. The disulfide band of rice globulin, from either fresh or aged rice, was centered at 497 cm^{-1}, suggesting that a possible monomer form exists in rice globulin in the solid state, which conflicts with the results reported by Ellepola et al. for

Table 2. Raman Frequencies and Tentative Assignments of Proteins from Fresh and Aged Rice

	band position (cm^{-1})			normalized intensity[a] (I/I_{1005})	
sample	fresh rice protein	aged rice protein	assignments	fresh rice protein	aged rice protein
albumin	480	482	starch skeleton	2.09 a	1.50 b
	510	510	S—S	0.74 b	1.00 a
	528	526	S—S	1.03 a	0.80 b
	749	749	Trp	0.64 a	0.65 a
	1334	1334	Trp	1.73 a	1.40 b
	856/830	850/832	Tyr	1.46 a	1.29 b
	933	936	C—C	0.90 a	0.83 a
	1256	1259	amide III, α-helix	1.17 a	0.99 a
	1662	1660	amide I, α-helix	1.23 a	1.19 a
	2934	2939	C—H	5.95 a	4.74 b
globulin		468	starch skeleton		1.02
		486	starch skeleton		0.86
	496	497	S—S	0.62 b	1.09 a
	513	513	S—S	0.57 b	0.80 a
	532	533	S—S	0.74 a	0.78 a
	546	547	S—S	0.77 b	1.03 a
	750	750	Trp	0.53 a	0.77 a
	1334	1335	Trp	0.84 b	1.11 a
	854/831	857/834	Tyr	1.08 a	1.20 a
		857	starch skeleton		1.24
		938	starch skeleton		0.88
	943	955	C—C	0.65 b	0.92 a
	1047	1041	S=O	0.79 b	1.58 a
	1070	1070	S=O	0.95 b	1.40 a
	1124	1128	O=S=O	0.68 b	1.09 a
	1148	1148	O=S=O	0.46 b	0.89 a
	1163	1163	O=S=O	0.51 b	1.00 a
	1271	1273	amide III	0.62 b	0.88 a
	1294	1298	O=S=O	0.73 b	1.17 a
	1316	1315	O=S=O	0.86 b	1.25 a
	1352	1351	O=S=O	0.60 b	1.19 a
	1438	1438	C—H	0.83 b	1.60 a
	1624	1622	β-sheet	0.67 b	1.74 a
	1656	1662	amide I	0.94 a	1.10 a
	2933	2925	C—H	2.95 a	2.55 a
glutelin	509	507	S—S	0.37 b	0.54 a
	1242	1246	amide III	0.72 a	0.63 a
	1666	1670	amide I	1.02 a	0.92 a
		2591	S—H		0.49
prolamin	501	508	S—S	0.72 a	0.85 a
	515	515	S—S	0.55 b	0.67 a
	532	528	S—S	0.74 a	0.68 b
	548	542	S—S	0.89 a	0.88 a
	852/828	851/827	Tyr	0.96 b	1.13 a
	1243	1220	amide III	0.70 a	0.67 a
	1674	1678	amide I	1.16 a	0.99 a

[a]Different letters (a, b) in the same row indicate significant differences ($p < 0.05$).

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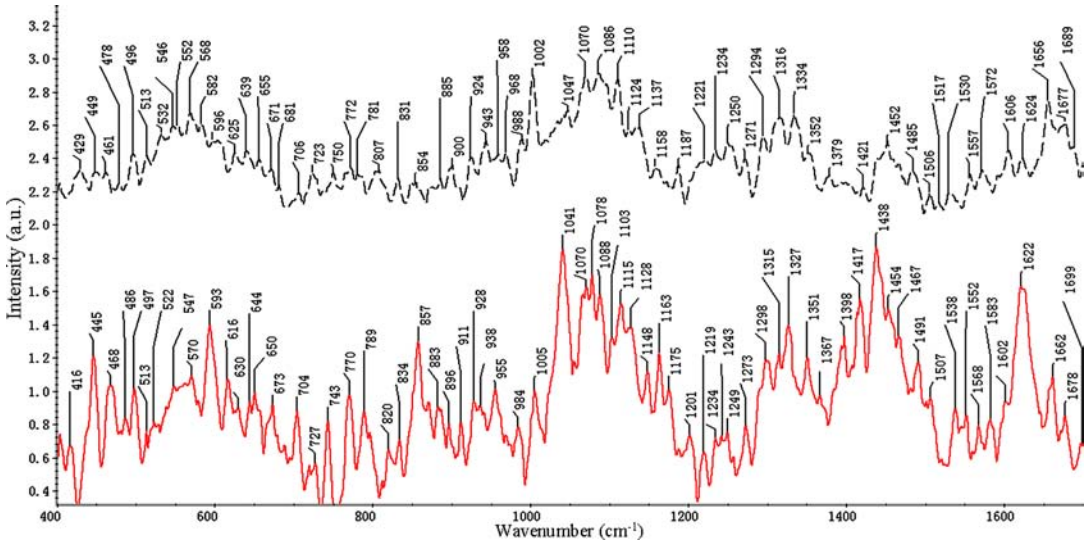

Figure 4. Raman spectra of globulins extracted from fresh rice (dashed line) and aged rice (solid line) in the 400−1700 cm^{-1} region.

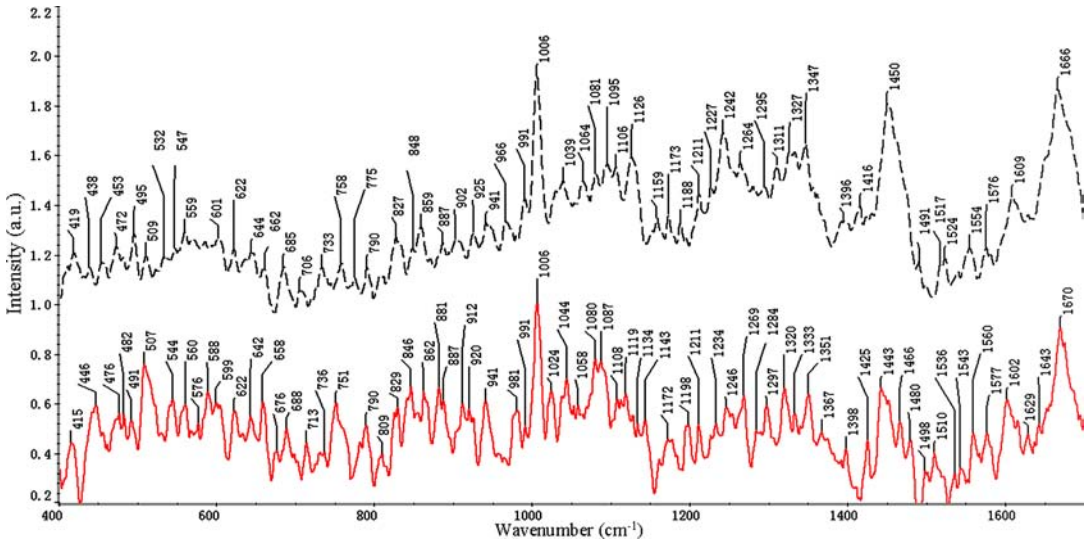

Figure 5. Raman spectra of glutelins extracted from fresh rice (dashed line) and aged rice (solid line) in the 400−1700 cm^{-1} region.

the solution state.[21] An increase in the intensity of the disulfide band near 497 cm^{-1} was clearly seen after rice aging. The bands near 513, 532, and 546 cm^{-1} represent the three different disulfide bond conformations.

The intensity of the globulin tryptophan band near 1334 cm^{-1} increased significantly, which indicates that more buried tryptophan residues were induced by rice aging.[21,36] It should be noted that this pattern of change was the opposite to that in albumin. The I_{850}/I_{830} intensity ratio of the tyrosine Fermi doublet in the Raman spectra of globulin did not change significantly with rice aging and was always found to be higher than 1.0. Therefore, tyrosine residues in rice globulin exist in the exposed state regardless of whether rice has been aged.

The Raman intensity of globulin C—H stretching bands near 2933 cm^{-1} was not influenced by rice aging. The maximum peak shifted from 2933 to 2925 cm^{-1} after rice aging, and more high-frequency shoulder peaks appeared. This indicates the diversity of the C—H conformation.

Sulfones (>SO_2) have strong characteristic bands at 1360−1290 and 1200−1120 cm^{-1}, whereas sulfoxides (R_2>S═O) are characterized by a strong infrared band and a moderate-to-weak Raman band between 1070 and 1030 cm^{-1} from the S═O stretch.[41] In Raman spectra of globulins, the intensities of the bands near 1351, 1315, 1298, 1163, 1148, and 1128 cm^{-1} all increased considerably (characteristic frequency range of sulfones). Combined with the increase in intensities of the bands near 1070 and 1041 cm^{-1} (characteristic frequency range of sulfoxides), this demonstrates oxidation of the sulfhydryl group in globulin after rice aging.

After rice aging, the characteristic frequency of starch was detected in the Raman spectrum of globulin, such as the bands near 938, 857, 486, and 468 cm^{-1},[22,31,33] which implies that there is an association between globulin and starch.

Raman Characterization of Structural Changes in Glutelin Induced by Rice Aging. Raman spectra of the glutelins from fresh and aged rice in the 400−1700 cm^{-1} region are shown in Figure 5.

Pronounced differences were seen in the glutelin spectra of fresh and aged rice. The amide I and III bands of glutelin from fresh rice were centered at 1666 and 1242 cm^{-1}, which clearly shows the predominance of the β-sheet structure.[33] In contrast, the amide I and III bands of glutelin from aged rice were

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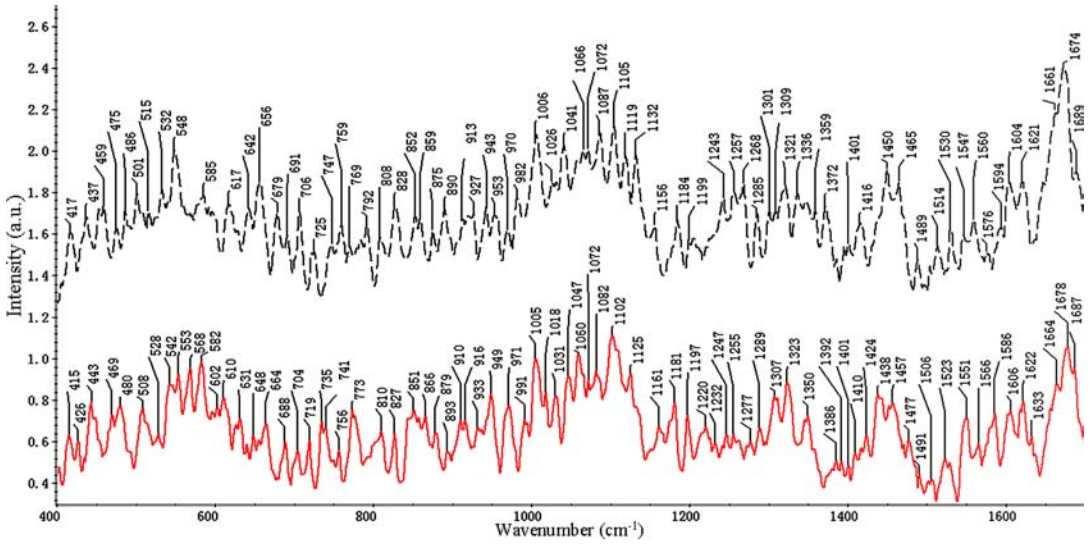

Figure 6. Raman spectra of prolamins extracted from fresh rice (dashed line) and aged rice (solid line) in the 400−1700 cm^{-1} region.

located at 1670 and 1246 cm^{-1}, which represent the antiparallel β-sheet.[21]

The disulfide band of rice glutelin from fresh and aged rice was primarily located at 509/507 cm^{-1}, which represents disulfide bonds in gauche−gauche−gauche conformations. An increase in intensity of the disulfide band near 507 cm^{-1} was clearly observed after rice aging. Furthermore, the peak near 2591 cm^{-1} in the Raman spectrum of glutelin appeared after rice aging, which was assigned to free sulfhydryl group.[34] The tryptophan, tyrosine doublet, and aliphatic C—H stretching vibration bands of glutelin did not change with rice aging.

Raman Characterization of Structural Changes in Prolamin Induced by Rice Aging. The Raman spectra of the prolamins from fresh and aged rice in the 400−1700 cm^{-1} region are shown in Figure 6.

The amide I and III bands of prolamin from fresh rice were located at 1674 and 1243 cm^{-1}. For aged rice, they were located at 1678 and 1220 cm^{-1}, which shows the predominance of β-sheets in prolamin.[33]

The disulfide bands of rice prolamin from fresh rice were located at 501, 515, 532, and 548 cm^{-1}, which represent disulfide bonds in the monomer and the three different conformations, respectively.[34,40] After rice aging, the disulfide bridge peak shifted from 501 cm^{-1} in monomer to 508 cm^{-1} in the dimer of gauche−gauche−gauche conformations.

Similarly to glutelin, the intensities of tryptophan and aliphatic C—H stretching vibrations of prolamin did not change with rice aging, but the intensity ratio of the tyrosine doublet was clearly altered. The ratio increased from 0.96 to 1.13 after rice aging (Table 2), which shows that the buried tyrosine residues became exposed as a result of rice aging.[36,38]

Among the four rice proteins, globulin changed the most with rice aging, followed by glutelin, and the structures of two other proteins also changed to varying degrees. These changes might be responsible for the difference in pasting properties between fresh and aged rice. Therefore, future research into the underlying mechanism of rice aging, in relation to structural changes in rice proteins, should be more focused on globulin rather than glutelin.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

FT-IR spectra of prolamins extracted from fresh rice (dashed line) and aged rice (solid line). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: kangtu@njau.edu.cn. Fax: +86-25-8439 6786. Tel.: +86-25-8439 9016.

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Notes

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■ REFERENCES

(1) Zhu, F.; Cai, Y.-Z.; Bao, J.; Corke, H. Effect of γ-irradiation on phenolic compounds in rice grain. *Food Chem.* **2010**, *120*, 74−77.

(2) Zhou, Z.; Robards, K.; Helliwell, S.; Blanchard, C. Ageing of Stored Rice: Changes in Chemical and Physical Attributes. *J. Cereal Sci.* **2002**, *35*, 65−78.

(3) Ramesh, M.; Bhattacharya, K. R.; Mitchell, J. R. Developments in Understanding the Basis of Cooked-Rice Texture. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*, 449−460.

(4) Gujral, H. S.; Kumar, V. Effect of accelerated aging on the physicochemical and textural properties of brown and milled rice. *J. Food Eng.* **2003**, *59*, 117−121.

(5) Zhou, Z.; Robards, K.; Helliwell, S.; Blanchard, C.; Baxterb, G. Rice Ageing. I. Effect of Changes in Protein on Starch Behaviour. *Starch/Stärke* **2003**, *55*, 162−169.

(6) Ohno, T.; Tomatsu, M.; Toeda, K.; Ohisa, N. Texture of Cooked Rice Prepared from Aged Rice and Its Improvement by Reducing Agents. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 2912−2920.

(7) Zhou, Z.; Robards, K.; Helliwell, S.; Blanchard, C. Effect of storage temperature on cooking behaviour of rice. *Food Chem.* **2007**, *105*, 491−497.

(8) Likitwattanasade, T.; Hongsprabhas, P. Effect of storage proteins on pasting properties and microstructure of Thai rice. *Food Res. Int.* **2010**, *43*, 1402−1409.

(9) Tananuwong, K.; Malila, Y. Changes in physicochemical properties of organic hulled rice during storage under different conditions. *Food Chem.* **2011**, *125*, 179−185.
(10) Zhou, Z.; Robards, K.; Helliwell, S.; Blanchard, C. Effect of storage temperature on rice thermal properties. *Food Res. Int.* **2010**, *43*, 709−715.
(11) Teo, C. H.; Karim, A. A.; Cheah, P. B.; Norziah, M. H.; Seow, C. C. On the roles of protein and starch in the aging of non-waxy rice flour. *Food Chem.* **2000**, *69*, 229−236.
(12) Chrastil, J. Protein−Starch Interactions in Rice Grains. Influence of Storage on Oryzenin and Starch. *J. Agric. Food Chem.* **1990**, *38*, 1804−1809.
(13) Lin, C. J.; Li, C. Y.; Lin, S. K.; Yang, F. H.; Huang, J. J.; Liu, Y. H.; Lur, H. S. Influence of high temperature during grain filling on the accumulation of storage proteins and grain quality in rice (*Oryza sativa* L.). *J. Agric. Food Chem.* **2010**, *58*, 10545−10552.
(14) Guo, Y.; Tu, K.; Pan, L.; Zhang, W.; Zhang, Y. Effects of three reducing agents on pasting properties of stored rice. *Starch/Stärke* **2012**, *64*, 198−206.
(15) Xie, L. N. C.; Duan, B.; Zhu, Z.; Liao, X. Impact of proteins on pasting and cooking properties of waxy and non-waxy rice. *J. Cereal Sci.* **2008**, *47*, 372−379.
(16) Martin, M.; Fitzgerald, M. A. Proteins in Rice Grains Influence Cooking Properties! *J. Cereal Sci.* **2002**, *36*, 285−294.
(17) Herrero, A. M.; Carmona, P.; López-López, I.; Jiménez-Colmenero, F. Raman Spectroscopic Evaluation of Meat Batter Structural Changes Induced by Thermal Treatment and Salt Addition. *J. Agric. Food Chem.* **2008**, *56*, 7119−7124.
(18) Petersen, F. N. R.; Nielsen, C. H. Raman Spectroscopy as a Tool for Investigating Lipid−Protein Interactions. *Spectroscopy* **2009**, *24*, 1−8.
(19) Dàvila, E.; Parés, D.; Howell, N. K. Studies on plasma protein interactions in heat-induced gels by differential scanning calorimetry and FT-Raman spectroscopy. *Food Hydrocolloids* **2007**, *21*, 1144−1152.
(20) Meng, G.; Chan, J. C. K.; Rousseau, D.; Li-Chan, E. C. Y. Study of Protein−Lipid Interactions at the Bovine Serum Albumin/Oil Interface by Raman Microspectroscopy. *J. Agric. Food Chem.* **2005**, *53*, 845−852.
(21) Ellepola, S. W.; Choi, S. M.; Phillips, D. L.; Ma, C. Y. Raman spectroscopic study of rice globulin. *J. Cereal Sci.* **2006**, *43*, 85−93.
(22) Piot, O.; Autran, J. C.; Manfait, M. Spatial Distribution of Protein and Phenolic Constituents in Wheat Grain as Probed by Confocal Raman Microspectroscopy. *J. Cereal Sci.* **2000**, *32*, 57−71.
(23) Ma, C.-Y.; Phillips, D. L. FT-Raman Spectroscopy and Its Applications in Cereal Science. *Cereal Chem.* **2002**, *79*, 171−177.
(24) Ma, C. Y.; Rout, M. K.; Chan, W. M.; Phillips, D. L. Raman spectroscopic study of oat globulin conformation. *J. Agric. Food Chem.* **2000**, *48*, 1542−1547.
(25) AACC Method 46-11A. Crude Protein—Improved Kjeldahl Method, Copper Catalyst Modification. In *AACC Approved Methods of Analysis*, 10th ed.; American Association of Cereal Chemists: St. Paul, MN, 2000.
(26) AACC Method 61-02. Determination of the Pasting Properties of Rice with the Rapid Visco Analyser. In *AACC Approved Methods of Analysis*, 9th ed.; American Association of Cereal Chemists: St. Paul, MN, 1995.
(27) Howell, N. K.; Arteaga, G.; Nakai, S.; Li-Chan, E. C. Y. Raman spectral analysis in the C−H stretching region of proteins and amino acids for investigation of hydrophobic interactions. *J. Agric. Food Chem.* **1999**, *47*, 924−933.
(28) Zhou, Z.; Robards, K.; Helliwell, S.; Blanchard, C. Effect of rice storage on pasting properties of rice flour. *Food Res. Int.* **2003**, *36*, 625−634.
(29) Larkin, P. J. *Infrared and Raman Spectroscopy: Principles and Spectral Interpretation*; Elsevier Inc.: Waltham, MA, 2011; p 35.
(30) Sánchez-González, I.; Carmona, P.; Moreno, P.; Borderías, J.; Sánchez-Alonso, I.; Rodríguez-Casado, A.; Careche, M. Protein and water structural changes in fish surimi during gelation as revealed by isotopic H/D exchange and Raman spectroscopy. *Food Chem.* **2008**, *106*, 56−64.
(31) Sivam, A. S.; Sun-Waterhouse, D.; Perera, C. O.; Waterhouse, G. I. N. Application of FT-IR and Raman spectroscopy for the study of biopolymers in breads fortified with fibre and polyphenols. *Food Res. Int.* **2011**, DOI: 10.1016/j.foodres.2011.03.039.
(32) Baxter, G.; Blanchard, C.; Zhao, J. Effects of prolamin on the textural and pasting properties of rice flour and starch. *J. Cereal Sci.* **2004**, *40*, 205−211.
(33) Sun, W.; Zhao, Q.; Zhao, M.; Yang, B.; Cui, C.; Ren, J. Structural evaluation of myofibrillar proteins during processing of Cantonese sausage by Raman spectroscopy. *J. Agric. Food Chem.* **2011**, *59*, 11070−11077.
(34) Li-Chan, E. C. Y. The applications of Raman spectroscopy in food science. *Trends Food Sci. Technol.* **1996**, *7*, 361−370.
(35) Byler, D. M.; Susi, H.; Farrell, H. M. Laser-Raman spectra, sulfhydryl groups, and conformation of the cystine linkages of β-lactoglobulin. *Biopolymers* **1983**, *22*, 2507−2511.
(36) Bouraoui, M.; Nakai, S.; Li-Chan, E. In situ investigation of protein structure in Pacific whiting surimi and gels using Raman spectroscopy. *Food Res. Int.* **1997**, *30*, 65−72.
(37) Li-Chan, E.; Nakai, S. Importance of Hydrophobicity of Proteins in Food Emulsions. In *Microemulsions and Emulsions in Foods*; El-Nokaly, M., Cornell, D., Eds.; ACS Symposium Series; American Chemical Society: Washington, D.C., 1991; Vol. *448*, pp 193−212.
(38) Shao, J.-H.; Zou, Y.-F.; Xu, X.-L.; Wu, J.-Q.; Zhou, G.-H. Evaluation of structural changes in raw and heated meat batters prepared with different lipids using Raman spectroscopy. *Food Res. Int.* **2011**, *44*, 2955−2961.
(39) Herrero, A. M. Raman spectroscopy a promising technique for quality assessment of meat and fish: A review. *Food Chem.* **2008**, *107*, 1642−1651.
(40) Nonaka, M.; Lichan, E.; Nakai, S. Raman Spectroscopic Study of Thermally Induced Gelation of Whey Proteins. *J. Agric. Food Chem.* **1993**, *41*, 1176−1181.
(41) Larkin, P. J. *Infrared and Raman Spectroscopy: Principles and Spectral Interpretation*; Elsevier Inc.: Waltham, MA, 2011; p 129.