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Thermal Stability and Molecular Microstructure of Heat-Induced Cereal Grains, Revealed with Raman Molecular Microspectroscopy and Differential Scanning Calorimetry

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ABSTRACT: The objectives of the present study were to use Raman molecular microspectroscopy and differential scanning calorimetry (DSC) to reveal molecular thermal stability and thermal degradation behavior of heat-induced cereal grains and reveal the molecular chemistry of the protein structures of cereal grain tissues affected by heat processing and to quantify the protein secondary structures using multicomponent peak modeling Gaussian and Lorentzian methods. Hierarchical cluster analysis (CLA) and principal components analysis (PCA) were also conducted to identify molecular differences in the Raman spectra. Three cereal grain seeds, wheat, triticale, and corn, were used as the model for feed protein in the experiment. The specimens were autoclaved (moist heating) and dry-heated (roasted) at 121 °C for 80 min, respectively. Raman spectroscopy results revealed that there are marked differences in the secondary structures of the proteins subjected to various heating treatments of different cereals. The sensitivity of cereals to moist heating was much higher than the sensitivity to dry heating. The multivariate analyses (CLA and PCA) showed that heat treatment was significantly isolated between the different Raman raw spectra. The DSC study revealed that the thermal degradation behavior of cereals was significantly changed after moist- and dry-heat treatments. The position of the major endothermic peak of dry-heated cereals shifted toward a higher temperature, from 131.7 to 134.0 °C, suggesting the high thermal stability of dry-heated cereals. In contrast, the endothermic peak position was slightly decreased to 132.1 °C in the case of moist autoclaved heating. The digestive behavior and nutritive value of rumen-undegradable protein in animals may be related to the changes of the protein secondary molecular structure and thermal stability of the cereal grain materials, which is attributed by Raman microspectroscopy and DSC endotherm profiles.

KEYWORDS: *Thermal stability, thermal degradation, heat treatment, protein fine structure*

INTRODUCTION

The nutritive value, degradation characteristics, utilization, and availability of protein are not only determined by the nutrient or chemical composition but also affected by intrinsic chemical structures, such as protein secondary structures and biological component matrix.[1−4] Therefore, an understanding of the molecular structure of the whole protein is often vital to understand its digestive behavior, nutritive quality, utilization, and availability in animals.[5−7] Protein secondary structures include mainly the α-helix and β-sheet, with small numbers of β-turns and random coils. The influence of such structures and the α-helix/β-sheet ratio on the protein quality, utilization, availability, and digestive behavior was important in feed evaluation.[2,3] The protein structure α-helix/β-sheet ratio affects the total intestinally absorbed protein supply (protein DVE value) and degraded protein balance (protein OEB value).[8−10]

Protein functionality is closely related to its conformational state, which, in turn, is completely influenced by processing conditions. The temperature is one of the most important factors in a process performance, because a high temperature causes protein unfolding and loss of functionality. Recent published reviews show that the effects of heat processing on the protein nutritive value and performance in animals are ambiguous.[11] Part of the reason is that heating conditions inside a feed may not be optimal, resulting in the feed being either underheated or overheated. In ruminants, heat processing has been used to improve the utilization and availability of nutrients[12−14] and inactivate antinutritional factors.[15] However, studies on protein secondary structures in relation to the nutritive value and digestive behaviors of protein in animals are extremely rare. Recently, Samadi and Yu[16] studied the dry and moist heating-induced changes in protein molecular structure, protein subfraction, and nutrient profiles in soybeans. They found that heat treatments changed the protein molecular structure.

Table 1. Tentative Peak Assignments of Deconvoluted Amide I Components

fine structure	amide I wavenumber (cm^{-1})
protein fine structure	
aggregated strands	ca. 1600−1620
β-sheet (low frequency)	ca. 1620−1640
β-sheet (high frequency)	ca. 1670−1680
β-turns	ca. 1680−1699
α-helix	ca. 1650−1660
random coil	ca. 1660−1670

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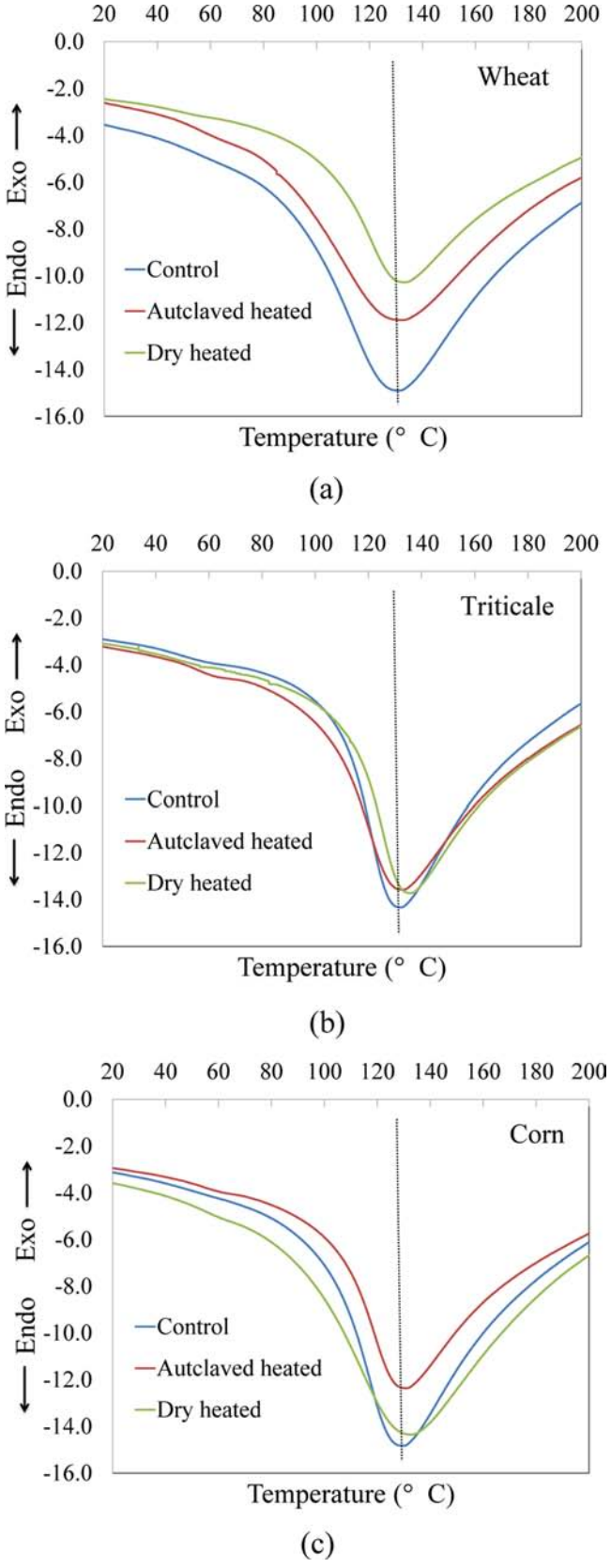

Figure 1. DSC curves of control, autoclaved, and dry-heated cereals: (a) wheat, (b) triticale, and (c) corn.

Both dry and moist heating increased the amide I/amide II ratio. The sensitivity of soybean seeds to moist heating was much higher than the sensitivity to dry heating in terms of the structure and nutrient profile changes. The autoclaved moist heating at 120 °C for different treatment times greatly influenced the molecular structure of Vimy flaxseed protein.[7] It has been showed that heating changed the chemical profile and protein subfractions, generally decreased ruminal degradability of protein, and increased potential nutrient supply. The protein secondary structure α-helix/β-sheet ratio had a positive correlation with the total intestinally absorbed protein supply and a negative correlation with the degraded protein balance.

Table 2. DSC Profiles of Heat-Induced Cereals: Comparison of Raw (Control) versus Dry-Roasted and Autoclaved Wheat, Triticale, and Corn: Effect of Heat Treatment[a]

item		property	
		T_d (°C)	ΔH_f (J/g)
cereal	wheat	132.7	122.1 b
	triticale	133.5	128.2 ab
	corn	131.5	139.2 a
	SEM	0.60	3.50
heating method	control	131.7 b	141.0 a
	dry-roasted	134.0 a	124.3 b
	autoclaved	132.1 ab	124.2 b
	SEM	0.60	3.50
statistical analysis	cereal (C)	0.065	0.004
	heating meathod (H)	0.026	0.001
	CH interaction	0.823	0.948
contrast	control versus heated	0.090	<0.001

[a]Control, raw without heating; dry, roasted at 121 °C for 80 min; and autoclaved, moist heated at 121 °C for 80 min. T_d = endothermic transition peak related to thermal decomposition of cereals. ΔH_f = enthalpy of fusion that indicates how much energy is needed to melt accompanying thermal decomposition for the cereals from their crystalline state. SEM = standard error of the mean. Means within a row with different letters differ ($p < 0.05$). Mean separation was performed using the Tukey–Kramer method.

Raman microspectroscopy is a powerful technique to study the microstructural characterization of the biopolymer, which provides information on the microenvironment and chemistry of protein side chains as well as the conformation of the protein polypeptide backbone.[17−21] Changes in Raman bands can give information on the secondary structures of proteins, particularly in the amide I and amide II region (1580−1700 cm^{-1}), which are due to contributions from the C═O stretching vibration of the amide group, coupled with the in-plane N−H bending and C−N stretching vibrations.[17−19] However, to our knowledge, there have been no Raman spectroscopic studies performed on animal feed materials processing under different heating conditions.

Differential scanning calorimetry (DSC) has been widely used to study protein thermodynamics, folding, and interactions.[22−24] It has also been used to characterize protein thermal stability, overall conformation, and domain folding integrity during formulation and product development by the biopharmaceutical industry.[25−27] It is very rare to use the DSC technique to evaluate the molecular structural features of animal feed materials related to their nutritive quality, utilization, and availability in animals.

In summary, the structural changes occurring in animal feed materials, such as different cereal grains, wheat, triticale, and corn, during heat-induced processing are not clearly understood. A better understanding of the structural changes of cereal proteins and carbohydrates occurring in feed products induced by thermal treatments could be helpful to elucidate

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Table 3. Percentage Breakdown of Different Fractions of Secondary Structures in Control, Dry-Heated, and Autoclaved-Heated Wheat, Triticale, and Corn Proteins

		percentage of secondary structures (%)					
		α-helix	β-sheet	ratio (α/β)	β-turns	random coil	aggregated strands
wheat	control	39.0	38.8	1.01	7.2	9.0	5.5
	dry-heated	44.5	39.2	1.14	8.0	0.0	8.7
	autoclaved	29.2	50.7	0.58	6.7	2.0	11.3
triticale	control	38.7	45.2	0.86	9.0	3.3	3.7
	dry-heated	60.7	28.0	2.17	2.8	5.3	3.2
	autoclaved	41.0	37.3	1.10	2.5	7.7	11.8
corn	control	47.3	35.3	1.34	5.2	0.0	12.3
	dry-heated	27.3	39.2	0.70	3.3	1.5	28.5
	autoclaved	25.0	38.8	0.64	1.5	4.3	30.5
	SEM	4.10	5.54	0.74	2.77	3.34	2.28
heating (overall)	control	41.7	39.8	1.05	7.1	4.1	7.2
	dry-heated	44.2	35.4	1.25	4.7	2.3	13.4
	autoclaved	31.7	42.3	0.75	3.6	4.7	17.9
	SEM	2.99	4.04	0.74	2.06	1.93	1.31
cereal (overall)	wheat	37.6	42.8	0.88	7.3	3.7	8.5
	triticale	46.8	36.8	1.27	4.8	5.4	6.2
	corn	33.2	37.8	0.88	3.3	1.9	23.8
	SEM	2.99	4.04	0.74	2.06	1.93	1.31
					p value		
statistical	cereal	<0.0001	0.2408	0.2047	0.1104	0.4445	<0.0001
	heating	0.0001	0.2025	0.0372	0.1599	0.6588	<0.0001
	cereal × heating	<0.0001	0.1067	0.0345	0.5871	0.3150	0.0051
					p value		
contrast	control versus heated	0.1323	0.7819	0.6076	0.0709	0.7878	<0.0001

their role in the protein matrix structure and for the development of new healthy animal feed products. The objectives of this study were to reveal the molecular chemistry of protein structures of the cereal grain affected by heat processing at the molecular level, using the Raman molecular spectroscopy and DSC techniques as a novel approach, and to quantify protein secondary structural data in relation to other studies on protein digestive behaviors of different cultivars.

MATERIALS AND METHODS

Experimental Materials. Three different types of cereal grains: wheat (CDC Go, Kermen Research Farm, Saskatoon, Saskatchewan, Canada, harvested at 2007), triticale (DeKalb RR DKC, Wright County, MN, harvested at 2011), and corn (AC Ultima, Lethbridge, Alberta, Canada, harvested at 2011) were used in the experiments. The cereals were provided by Crop Development Center, University of Saskatchewan, Canada and Lethbridge Research Center, Agriculture and Agri-Food Canada, Canada.

Heat Treatment and Processing. A 2 kg sample of each cereal was heated by moist heating (autoclaving) and dry heating (Amsco Eagle SG-3031, Steris Corp., Mentor, OH) for 80 min at 121 °C. The temperature and duration was chosen based on the preliminary study by Mustafa et al.[28] The treatment was performed in two batches as replication. Control samples were kept untreated. Heated samples were subsequently cooled at room temperature (20−22 °C) and then placed in the refrigerator to minimize sticking and clumping while grinding. The samples were ground using a Retsch SM 2000 (Retsch, Inc., Newtown, PA) fitted with a 2 mm screen. Samples were fed slowly into the grinder to further prevent sticking and clumping during the grinding process and to ensure that the seed was cracked open and not "extruded" through the screen.

Raman Molecular Spectroscopy. Raman spectroscopy measurements were carried out using a Renishaw InVia Raman microscope equipped with a solid-state laser diode (Renishaw) operating at 785 nm and a 1200 lines/mm grating. The microscope was focused on the sample using a Leica 20× N PLAN objective lens (NA = 0.40), and backscattered Raman signals were collected with a Peltier-cooled charge coupled device (CCD) detector. The instrument was operated in the line focus confocal mode at a 32 s detector exposure time with 32 spectra accumulations. The laser power was set at 0.1% (>300 mW measured at the output aperture of the laser). The instrument was calibrated using an internal Si sample, which was measured at 520 cm^{-1}. The spectra were recorded in the range of 400−3600 cm^{-1}. The Raman spectral data of each area were analyzed using OMINIC 7.2 (Spectra-Tech, Madison, WI) software. Chemical functional groups were identified according to previous literature.[29−31] To calculate the secondary structure components, the amide I region (1600−1700 cm^{-1}) was truncated and deconvoluted using a nonlinear least-squares curve-fitting subroutine, which included mixed Gaussian and Lorenztian components. The tentative peak assignments of deconvoluted amide I components were performed according to published literature,[32] as shown in Table 1. The percentage of each secondary structure component, that is, α-helix, β-sheet, β-turns, and random coil, was determined by the following equation:[33]

$$\text{percentage of secondary structure} = \frac{\text{area of secondary structure}}{\text{area of amide I band}} \times 100 \quad (1)$$

DSC. The DSC measurement was performed by a DSC Q2000 modules instrument (Q Series Thermal Analysis, TA Instruments, New Castle, DE) at a heating rate of 10 °C min^{-1} under a N_2 gas atmosphere. The endothermic transition peak (T_d) related to thermal decomposition of cereals was determined as the peak value of the endothermal phenomenon in the DSC curve. The enthalpy of fusion (ΔH_f), which indicates how much energy is needed to melt accompanying thermal decomposition for the cereals from their crystalline state, was determined from the total area of the DSC endotherm.

Statistical Analysis. The statistical analysis of Raman spectra and DSC analysis were performed using the MIXED procedure of SAS software.[34] The model used for analysis was as follows:

$$Y_{ij} = \mu + F_i + P_j + F_iP_j + e_{ijk}$$

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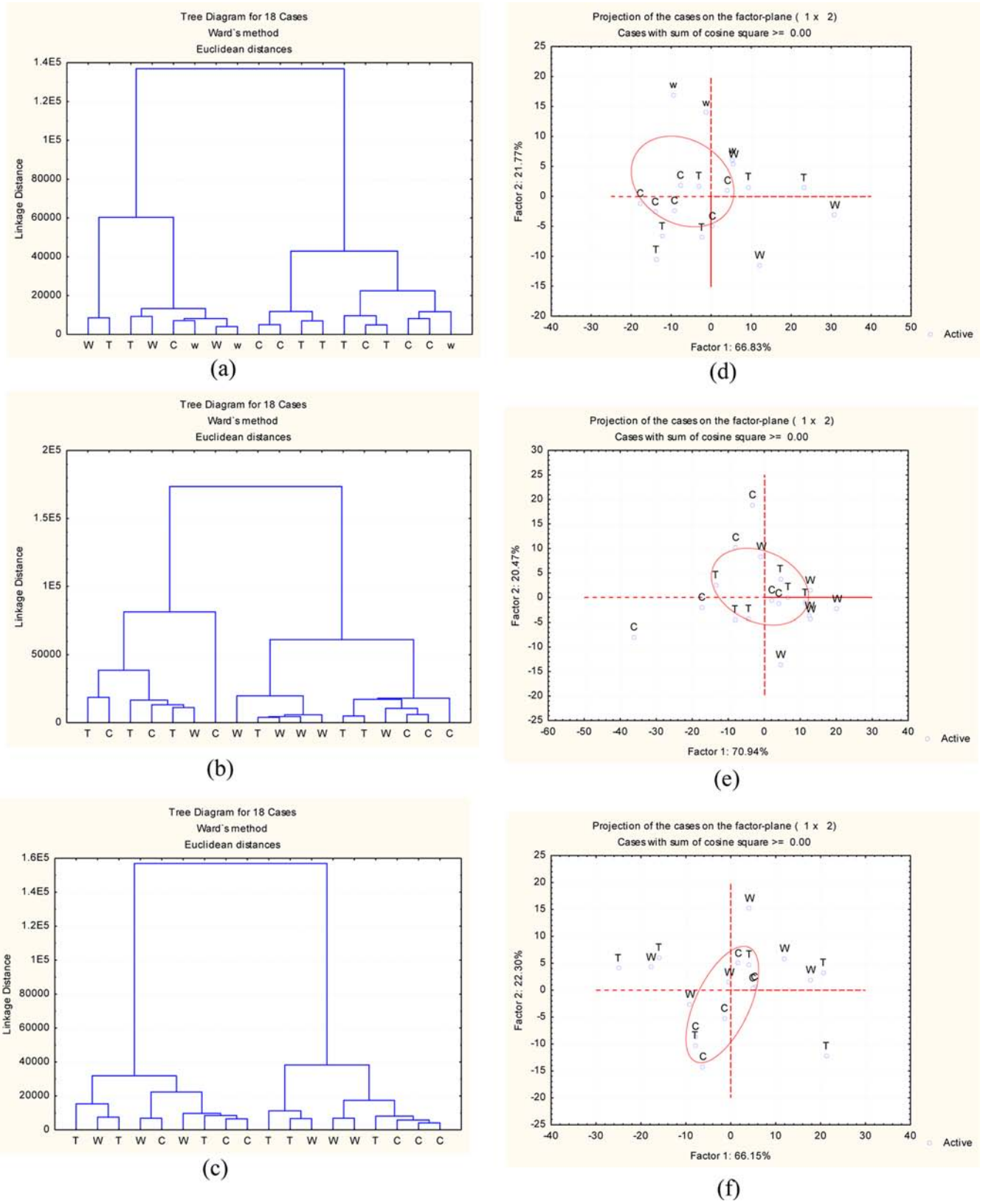

Figure 2. Multivariate analysis of Raman spectra (ca. 2000−800 cm^{-1}) obtained from (a and d) control (without heating); (b and e) dry-roasted (at 121 °C for 80 min), and (c and f) autoclaved (moist heated at 121 °C for 80 min) cereals (W, wheat; T, triticale; and C, corn). (a−c) CLA and (d−f) PCA.

where Y_{ij} is an observation on the dependent variable ij, μ is the population mean for the variable, F_i is the effect of cereal seed variety as a fixed effect, P_j is the effect of heat treatment as a fixed effect, F_iP_j is the interaction between cereal grains and processing methods, and e_{ijk} is the random error associated with the observation ijk. Batches were processed as replications. When a significant difference was detected ($p < 0.05$), means were separated using the Fisher's protected least significant difference test.

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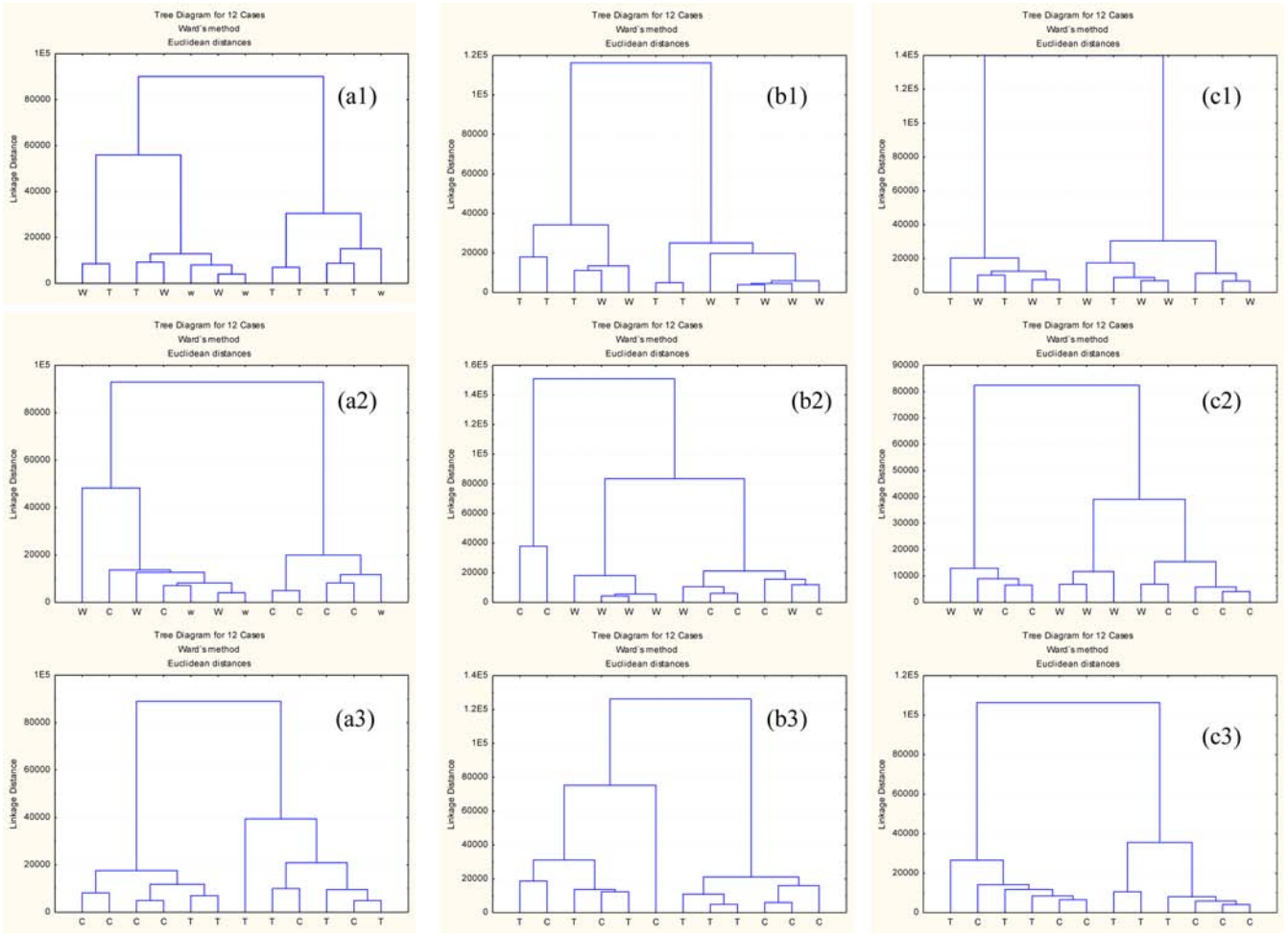

Figure 3. CLA of Raman spectra (ca. 2000−800 cm^{-1}) obtained from (a) control (without heating), (b) dry-roasted (at 121 °C for 80 min), and (c) autoclaved (moist heated at 121 °C for 80 min) cereals. Comparisons of different cereals at different treatments (1, W versus T; 2, W versus C; and 3, C versus T) (W, wheat; T, triticale; and C, corn).

Multivariate Analysis of Protein Spectra. The multivariate methods of data analysis were used to classify spectral groups by applying the whole spectral information. The multivariate analysis included agglomerative hierarchical cluster analysis (CLA), using Wards' algorithm method without prior parametrization, and principal component analysis (PCA), which was performed using Statistica software 8.0 (StatSoft, Inc., Tulsa, OK). For the purpose and objectives of this study, statistical comparisons were performed within grain types (wheat, triticale, or corn) and heating conditions (control, dry, and autoclaved) and results were presented under the same theme.

RESULTS AND DISCUSSION

Thermal Degradation of Cereal Grain Materials Affected by Heating Processing. The structural stability of cereal grain materials, such as wheat, triticale, and corn, was evaluated on the basis of the DSC measurement. Figure 1 shows the DSC thermograms of different cereals at different heating conditions. The results are summarized in Table 2. The different types of cereals show different endothermic transition peaks and enthalpies of fusion. The DSC curve of different cereals is characterized by a single predominant peak at 132.7, 133.5, and 131.5 °C for wheat, triticale, and corn, respectively, which is attributed to the thermal decomposition of cereals. Accordingly, the value of ΔH_f for wheat, triticale, and corn was 122.1, 128.2, and 139.2 J/g, respectively. The thermal decomposition of cereals was not significantly different, whereas heat enthalpy was remarkably different for the three types of cereals. The standard error of the mean (SEM) value was higher for ΔH_f in comparison to that of T_d.

The thermal degradation behavior of cereals was significantly changed after moist- and dry-heat treatments. The position of the major endothermic peak of cereals at control, dry, and moist autoclaved heating conditions was 131.7, 134.0, and 132.1 °C, respectively. It is observed that the endothermic peak position was shifted toward a higher temperature from the control (131.7 °C) to dry heating (134.0 °C), suggesting the high thermal stability of dry-heated cereals. In contrast, the endothermic peak position was decreased to 132.1 °C in the case of moist autoclaved heating, which is comparable to the non-heating control condition. These results strongly support the published report regarding DSC analysis of wheat cooking.[35] Stapley et al.[35] found that the DSC transition peak position depends upon the moisture content of samples. In general, the water content causes a shifting endothermic peak at a lower position. Because autoclaved heating was performed in the moist atmosphere, the endothermic transition was shifted at a lower temperature. It has been reported that the sensitivity of soybean seeds to moist heating was much higher than the sensitivity to dry heating in terms of the structure and nutrient profile changes.[16] They showed that moist heating decreased protein rumen degradability and increased intestinal digestibility of

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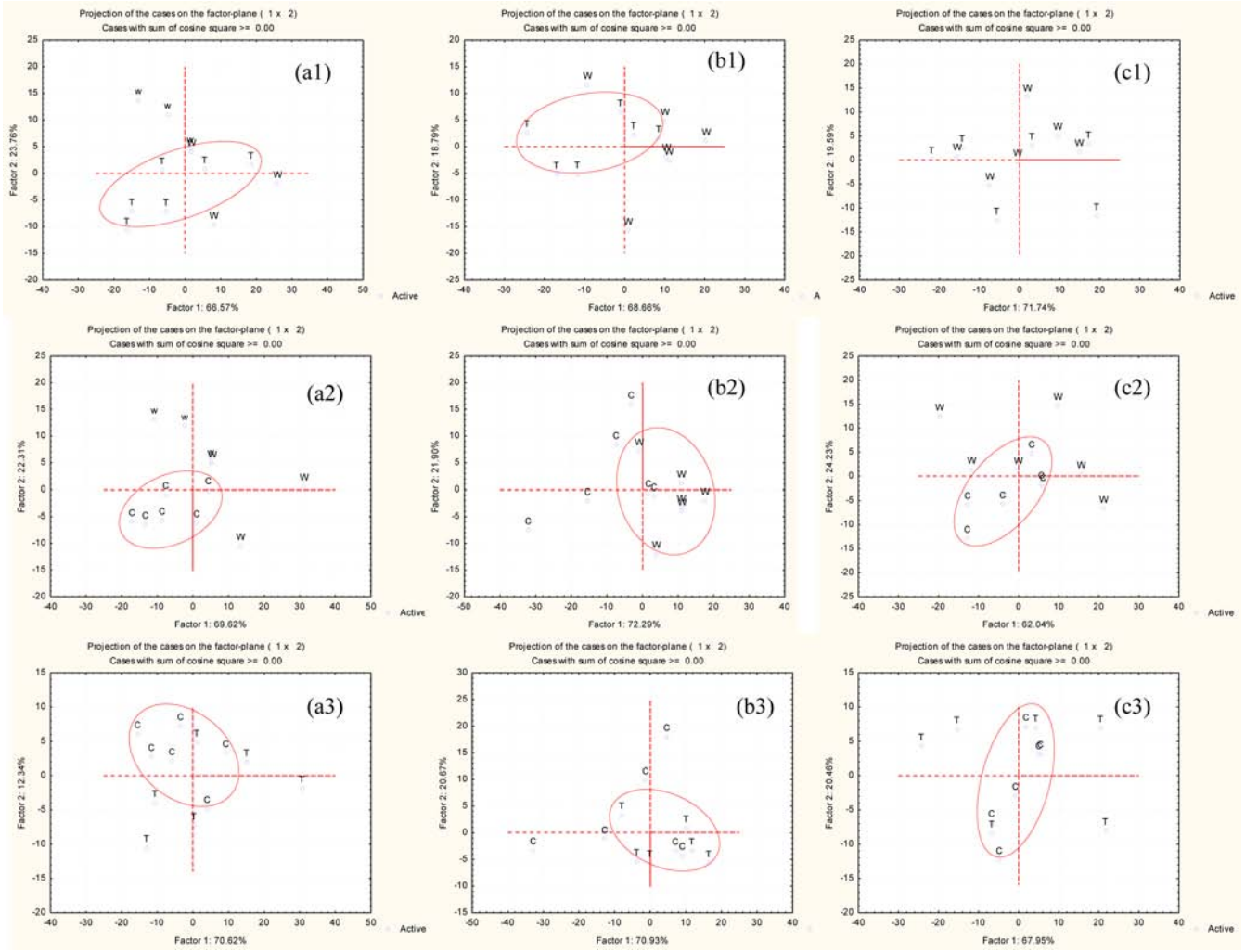

Figure 4. PCA of Raman spectra (ca. 2000−800 cm^{-1}) obtained from (a) control (without heating), (b) dry-roasted (at 121 °C for 80 min), and (c) autoclaved (moist heated at 121 °C for 80 min) cereals. Comparisons of different cereals at different treatments (1, W versus T; 2, W versus C; and 3, C versus T) (W, wheat; T, triticale; and C, corn).

rumen-undegradable protein. The increase of intestinal digestibility of rumen-undegradable protein may be related to the thermal stability of the materials, which is attributed by DSC endotherm profiles.

Molecular Structural Changes by Raman Spectra. The inherent protein structures influence the protein access to gastrointestinal digestive enzymes. Different α-helix/β-sheet ratio profiles affected the feed protein access to intestinal digestive enzymes.[7] Even if tissues contain the same protein content, their nutritive value may be different if their α-helix/β-sheet ratios in their protein secondary structures are different. It has been reported that heat treatments changed the protein molecular structure of soybean protein. Both dry and moist heating increased the amide I/amide II ratio. However, for the protein α-helix/β-sheet ratio, moist heating decreased but dry heating increased the ratio.[16] To evaluate the influence of heat treatment on cereal feeds, we studied the percentage of protein secondary structure of wheat, triticale, and corn after moist and dry heating. The results of deconvulated amide I bands of control, dry, and autoclaved heat-treated cereals are shown in Table 3. There are marked differences in the secondary structures of the proteins subjected to various heating treatments of different cereals. Quantitative analysis revealed that control wheat had a composition corresponding to 39.0% α-helix, 38.8% β-sheet, 7.2% β-turns, 9.0% random coil, and 5.5% aggregated strands. The ratio of α-helix/β-sheet was 1.01. After dry- and moist-heat treatments, wheat protein compositions were changed to 44.5% α-helix, 39.2% β-sheet, 8.0% β-turns, and 8.7% aggregated strands for dry-heat treatments and 29.2% α-helix, 50.7% β-sheet, 6.7% β-turns, 2.0% random coil, and 11.3% aggregated strands for autoclaved moist-heat treatments, respectively. The ratio of α-helix/β-sheet was 1.14 and 0.58 for dry- and moist-heat treatments, respectively. Accordingly, the secondary structures of triticale and corn also remarkably changed after dry- and moist-heat treatments. The alteration in the protein structure compositions and the ratio of α-helix/β-sheet was probably caused by denaturation of α-helix and β-sheet during the heating process. These results are consistent with the change of the protein structures of flaxseed tissues at different conditions reported by Doiron et al.[7]

Multivariate Molecular Analysis. CLA and PCA are two multivariate analyses that could be used for IR spectrum analysis.[36] The multivariate analysis, CLA, and PCA of Raman spectra for heat-treated cereal feeds were performed to discriminate and classify inherent structures between and among feed structures and feed molecular chemistry. Figure 2 shows the

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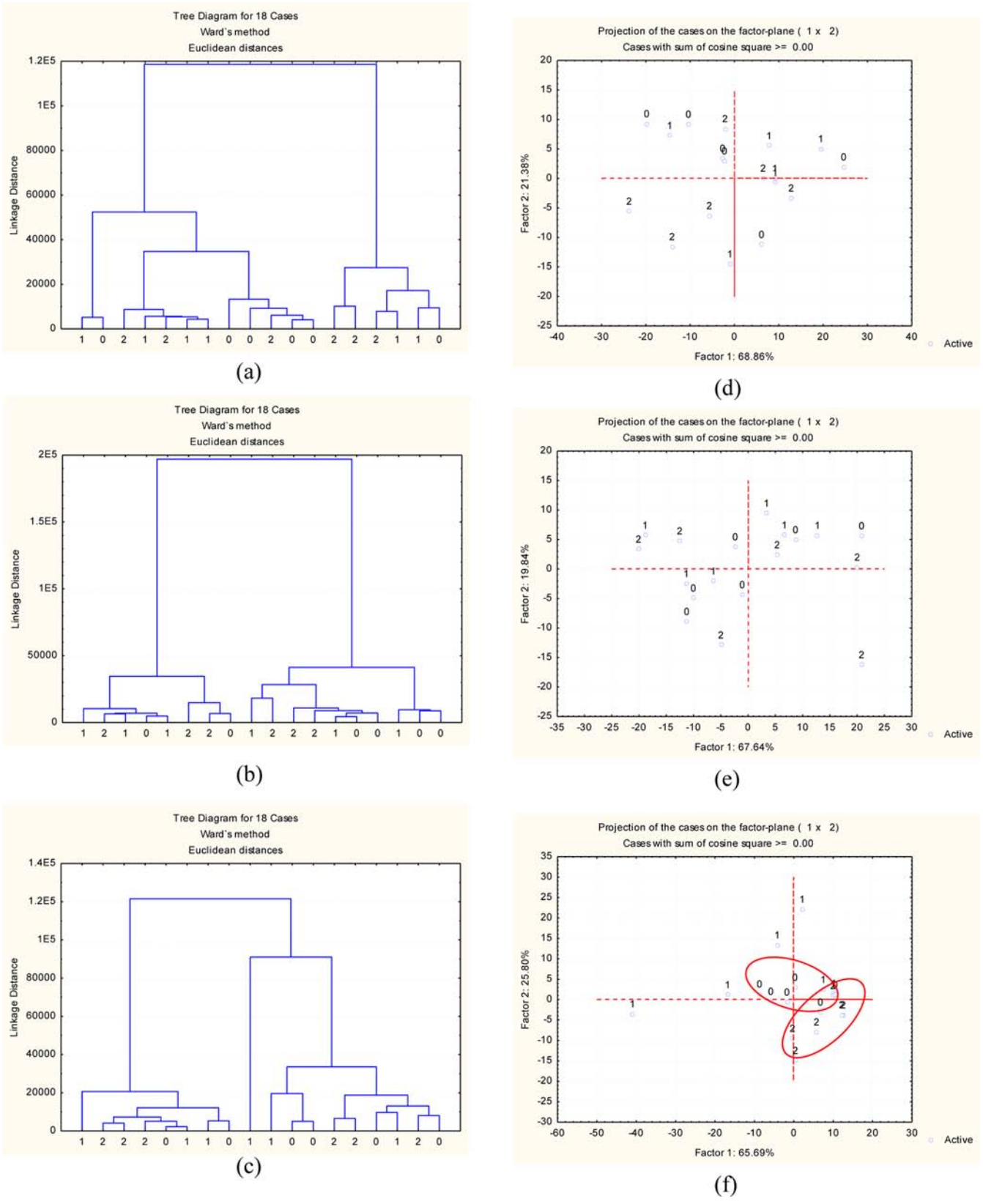

Figure 5. Multivariate analysis of Raman spectra (ca. 2000−800 cm^{-1}) for different cereals: (a and d) wheat, (b and e) triticale, and (c and f) corn [0, control (without heating); 1, dry roasted (at 121 °C for 80 min); and 2, autoclaved (moist heated at 121 °C for 80 min)] (a−c) CLA and (d−f) PCA.

results of CLA and PCA of wheat, triticale, and corn at different heating conditions for the ca. 2000−800 cm^{-1} spectral region based on Raman spectra. The cereals were in combined clusters at control conditions, and heat treatments did not show any significant individual cluster group (panels a−c of Figure 2). PCA results revealed that the three cereals were scattered,

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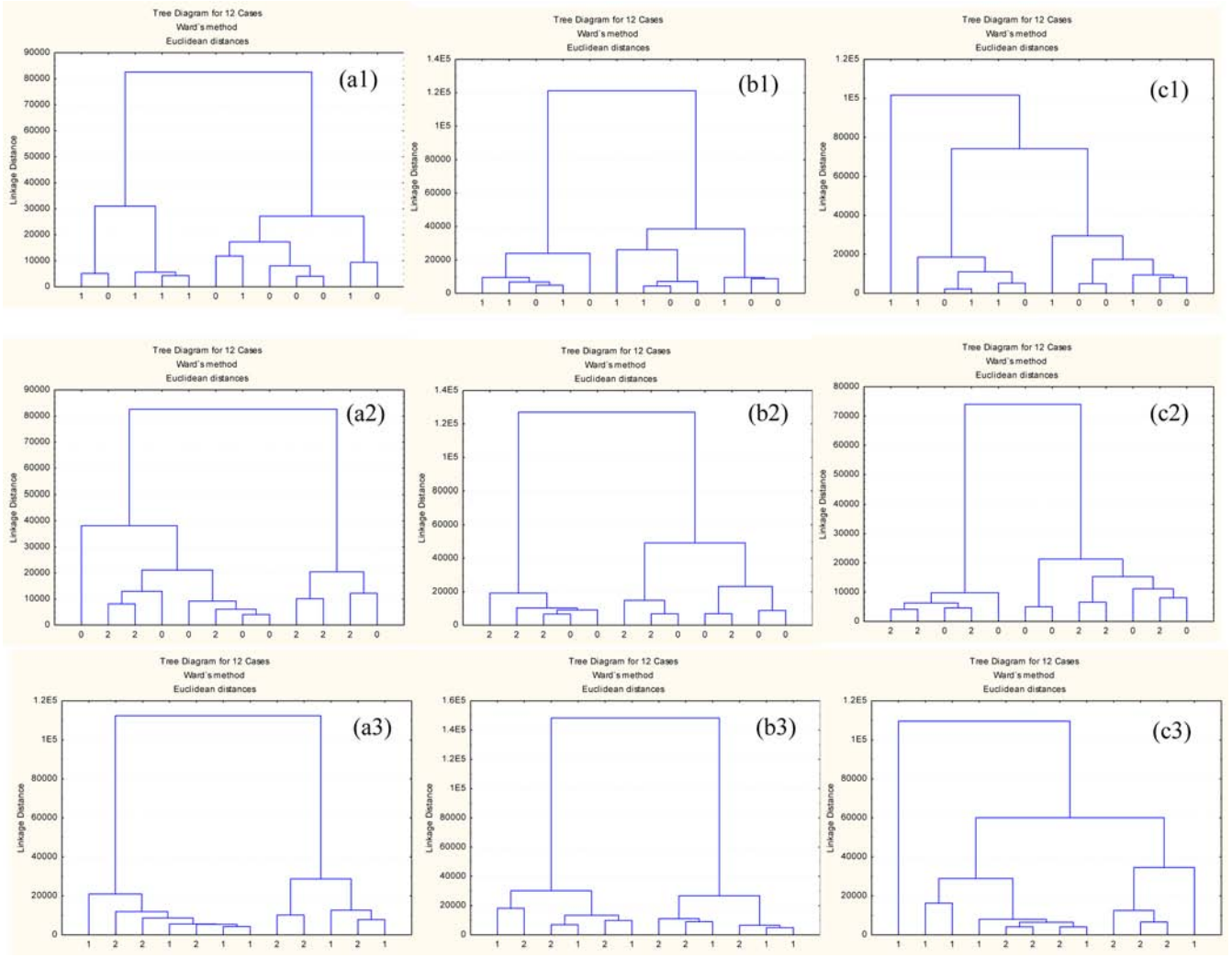

Figure 6. CLA of Raman spectra (ca. 2000−800 cm^{-1}) for different cereals: (a) wheat, (b) triticale, and (c) corn. Comparisons of different cereals at different treatments (1, 0 versus 1; 2, 0 versus 2; and 3, 1 versus 2) [0, control (without heating); 1, dry roasted (at 121 °C for 80 min); and 2, autoclaved (moist heated at 121 °C for 80 min)].

although corn showed unique groups at control (Figure 2d) and autoclaved-heating (Figure 2f) conditions and triticale was grouped in almost separate ellipses after dry heating (Figure 2e).

The comparison by CLA and PCA of each cereal wheat, triticale, and corn at different heating conditions for the ca. 2000−800 cm^{-1} spectral region based on Raman spectra was shown in Figures 3 and 4, respectively. The cluster analysis comparisons show a significantly isolated cluster group of each cereal at different heating conditions. Triticale was grouped separately from wheat in the control (Figure 4a1) and after dry heating (Figure 4b1). Corn showed perfectly separation from wheat in control samples (Figure 4a2), whereas it was overlapped in both dry and autoclaved heating (panels b2 and c2 of Figure 4). Triticale and corn spectral comparisons (panels a3−c3 of Figure 4) were not able to show any clear grouping of the spectra on the factor plane.

To evaluate the effects of heating treatments in the case of every individual cereal feeds, CLA and PCA of control and dry- and autoclaved-heated cereals were carried out for the same Raman spectra region, ca. 2000−800 cm^{-1}. The results show that the raw spectra were mixed up and did not isolate in their own spectra (Figure 5). For corn, the raw spectra for control and autoclaved specimens were shown as an individual group (Figure 5). The comparison results between two treatments, dry and autoclaved heating, showed that autoclaved-heating data were more isolated from control data in comparison to the dry-heating condition (Figures 6 and 7). Yu[3] reported that both PCA and CLA methods gave satisfactory analytical results and are conclusive in showing that they can discriminate and classify inherent structures and molecular chemistry between and among the feed tissues. They also can be used to identify whether differences exist between the varieties. Liu and Yu[13] applied CLA and PCA to distinguish different genotypes of barley. Recently, Abeysekara et al.[37] revealed that characterization of the protein molecular structure using non-invasive and non-destructive Fourier transform infrared (FTIR) spectroscopy as a novel approach with univariate and multivariate molecular spectral analyses yielded positive results, proving the ability of dried distiller's grains with solubles (DDGS) to impose intrinsic molecular and structural chemical changes in other grains, barley, corn, and oat. From the above CLA and PCA results, it might conclude that corn seed with autoclaved heating is more effective among three cereals, wheat, triticale, and corn, at control and dry- and autoclaved-heat treatments.

In conclusion, the different cereal grains, wheat, triticale, and corn, were heated at dry- and moist-heating conditions and their intrinsic molecular structural features and thermal

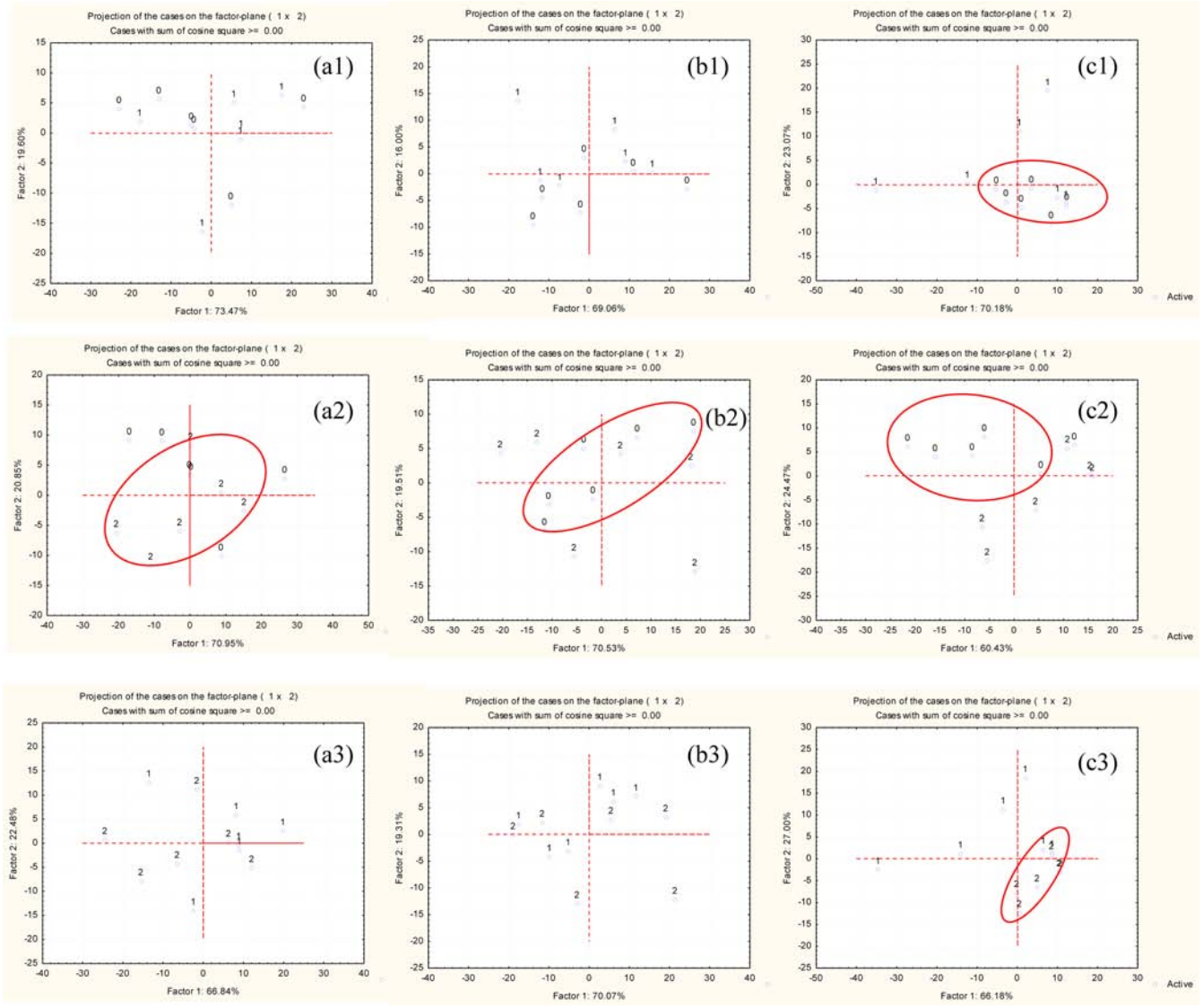

Figure 7. PCA of Raman spectra (ca. 2000−800 cm^{-1}) for different cereals: (a) wheat, (b) triticale, and (c) corn. Comparisons of different cereals at different treatments (1, 0 versus 1; 2, 0 versus 2; and 3, 1 versus 2) [0, control (without heating); 1, dry roasted (at 121 °C for 80 min); and 2, autoclaved (moist heated at 121 °C for 80 min)].

stabilities were evaluated in this study. The sensitivity of cereals to moist heating was much higher than the sensitivity to dry heating in terms of the structure and nutrient profile changes. The increase of intestinal digestibility of rumen-undegradable protein may be related to the thermal stability of the materials, which is attributed by DSC endotherm profiles.

Raman spectroscopy results revealed that there are marked differences in the secondary structures of the proteins subjected to various heating treatments of different cereals. Both PCA and CLA methods gave satisfactory results and are conclusive in showing that they can discriminate and classify inherent structures between feed structures, which may be highly associated with feed quality and nutritive value and digestive behaviors in animals. Further investigation of the influence of molecular thermal stability and degradation affected by various heat-processing conditions in relation to degradability and digestibility is required in feed materials.

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Notes

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