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Rumen Microbial Degradation of Beet Root Pulps. Application of Infrared Spectroscopy to the Study of Protein and Pectin

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Beet root pulps were degraded in the rumens of fistulated goats, by the nylon bag technique. A total of 36 residues were obtained, and their spectra were collected between 1400 and 2000 cm^{-1}. Proteins and pectins in this area present characteristic absorption bands. Principal-component analysis was applied to the spectral data in order to obtain a similarity map and spectral patterns. The absorption band of the normalized spectra at 1740 cm^{-1} decreased when the pulps were degraded. This result showed that pectins were highly digestible. The evolution of the amide II band expressed that the protein content of the residues increased when the pulps were degraded. A discrimination of the residues according to the degradation was observed on the similarity map obtained by principal-component analysis. The samples could also be classified according to the protein and the galacturonic acid contents on this map. The spectral patterns of proteins and pectins showed that the broad band at 1645 cm^{-1} could be split up into amide I bands and a peak corresponding to galacturonic acids.

Beet pulps are the main solid byproducts of the sugar industry and are commonly used as feed for ruminants. They are highly digestible and are an interesting source of metabolizable energy: The digestibility of the organic matter is about 80% for the ruminant (Jarrige, 1978). The pulps are composite materials consisting of cellulose (20% DM), hemicellulose (25% DM), pectic substances (25% DM), and protein (10% DM). The pectins are made of a main chain of polygalacturonic acid and of ramifications of L-arabinose and D-galactose. Several authors have studied the ruminal degradation of pectins of various feedstuffs. Gaillard (1962) originally showed the high fermentability of pectins. Chesson and Monro (1982) have investigated the relationship between the degree of acetylation and esterification of galacturonans of legumes and their degradation rates in the rumen. Sauvant et al. (1985) have applied the Van Soest analytical procedure to the study of various concentrates and byproducts, including beet pulps. The intent of the Van Soest method is to separate the highly available fractions from those that are less digestible. The pectins are not isolated and cannot be studied using this method. Theander and Aman (1980) have shown that pectins can be extracted either from the neutral detergent fiber (NDF) or from the acid detergent fiber (ADF) residues. Ben-Ghedalia and Rubinstein (1984) and Ben-Ghedalia and Miron (1984) have applied a colorimetric technique for quantifying galacturonic acid in oat, vetch hays, and alfalfa.

The aim of the present work is to investigate infrared spectroscopy in the study of the degradation of beet pulps in the rumens of fistulated goats. The spectra were collected in the middle infrared area, between 1400 and 2000 cm^{-1}, where both proteins and pectins have significant absorption bands. In this spectral range, the peptide bonds have distinct vibrational modes, expressed as amide I and amide II bands. The main contribution to the amide I band is the in-plane peptide carbonyl stretching vibration. The amide II band corresponds to a mixture of C–N stretch and N–H in-plane bend (Zundel et al., 1984). The polygalacturonic acids are partly methylated or ionized and present absorption bands at 1745 cm^{-1} (C=O ester) and 1608 cm^{-1} (COO^-) (Filippov and Kohn, 1975; Filippov et al., 1978). Given that certain significant bands of proteins

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and pectins are overlapping, the corresponding spectral information can only be extracted by mathematical processes. In the present work, principal-component analysis (PCA) was applied to achieve spectral decomposition. PCA had been previously applied to near- and mid-infrared spectra of food products. Bertrand et al. (1987) used PCA to predict protein content and in vitro dry matter digestibility of lucerne forage. Isaksson and Naes (1987) showed that this method compares favorably with other mathematical techniques for near-infrared predictions. Cowe and McNicol (1985) investigated PCA as a means to achieving spectral decomposition. In the present paper, PCA enabled similarity maps of the spectra and spectral patterns to be obtained.

MATERIALS AND METHODS

Samples. A total of 36 samples were obtained from the microbial degradation of beet pulp with use of the nylon bag technique initially described by Quin et al. (1938) and revised by Sauvant et al. (1985). Three mature nonlactating goats were used. Each animal was fitted with a rumen cannula (60-mm internal diameter). The goats were kept in individual pens and fed with hay and a concentrate blend (soybean meal 11%; maize grain 65%; maize oil 5%; cerelose 9.5%; urea 3%; mineral and vitamin mixture 6.5%). The beet pulps were ground in a hammer mill with a 2.0-mm screen. A 5-g portion of the product was then put into each nylon bag. After a wetting period of 30 min in water, the bags were introduced through the cannula into the rumen. They were then removed, washed with tap water, and freeze-dried after ruminal incubation times of 1, 4, 8, 16, and 48 h.

Analytical Methods. The percentage of degradation of the dry matter was obtained gravimetrically. The protein content of the residues (percent dry matter) was determined by the Kjeldahl method (AOAC, 1975). The pectic substances were assessed by measuring the galacturonic acid content (percent dry matter) in hydrolysates. The galacturonic acids were determined by the automated *m*-phenylphenol method (Thibault, 1979).

Spectroscopic Measurements. The infrared spectra were collected between 1400 and 2000 cm^{-1} in 4-cm^{-1} increments, using a Fourier transform spectrometer (Nicolet 10 DX). The apparatus was fitted with a TGS detector. Transmittance measurements of the samples were made on pressed pellets. The pellets were prepared by pressing a mixture of KCl and product at a concentration of 3% (w/w). In order to reach the signal to noise ratio, 400 scans were averaged for each spectrum. Before each spectrum was recorded, the spectrometer was dried with air for 5 min.

Mathematical Treatments. The suggested mathemetical processing was tested on an IBM-AT microcomputer. The software had been developed in the authors' laboratory and permitted both the normalization of the spectra and the application of PCA.

Normalization. Differences between the general intensities of the spectra are due to variations in the conditions of spectral recordings. To eliminate this artifact, the spectra were normalized according to the equation

$$n_i = d_i/(\sum_i d_i^2)^{1/2} \quad (1)$$

where d_i = optical density at the wavenumber i and n_i = normalized spectral data at the wavenumber i.

Similarity Maps. PCA on spectral data were achieved in order to obtain maps that showed resemblances and differences among spectra. These maps were obtained by selecting couples of principal components. On these graphs, the spectra were placed according to their principal-component scores. The scores were defined by

$$\mathbf{C} = \mathbf{X}\cdot\mathbf{V} \quad (2)$$

where $\mathbf{C}$ = rectangular matrix of scores, $\mathbf{X}$ = matrix of the spectral data, and $\mathbf{V}$ matrix of the eigenvectors of $\mathbf{X}'\mathbf{X}$ ($\mathbf{X}'$ = transposed matrix of $\mathbf{X}$).

Spectral Patterns. It was possible to compile the table of the spectral data $\mathbf{X}$ by knowing $\mathbf{V}$ and $\mathbf{C}$

$$\mathbf{X} = \mathbf{C}\cdot\mathbf{V}' \quad (3)$$

Table I. Protein and Galacturonic Acid Contents (Percent Dry Matter) of the Residues

constituent	range	mean	std dev
protein	8.83–18.20	12.23	2.19
galacturonic acid	4.62–19.20	15.40	3.54

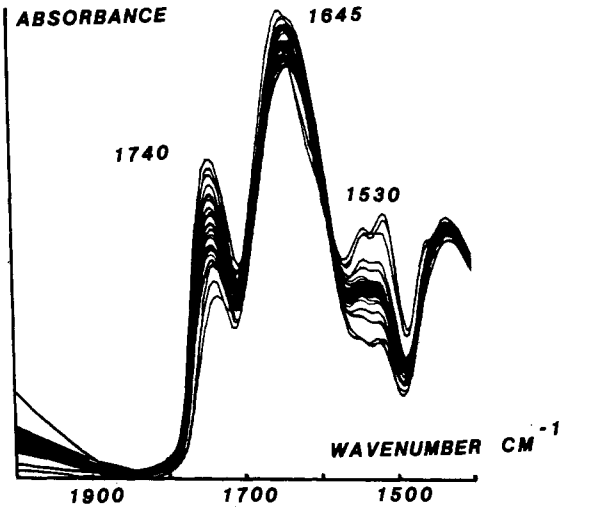

Figure 1. Normalized spectra of nylon bag residues.

where $\mathbf{V}'$ = transposed matrix of $\mathbf{V}$. Equation 3 gives for an observation x_i

$$x_i = c_{i1}\cdot v_1 + c_{i2}\cdot v_2 + \ldots + c_{ia}\cdot v_a \quad (4)$$

where $c_{i1}, c_{i2}, \ldots, c_{ia}$ = scores of the observation x_i, $v_1, v_2, \ldots, v_a$ = corresponding eigenvectors, and a = number of principal components.

Equation 4 shows that PCA splits a spectrum into a sum of signals. The proportions of the signals are given by the scores. This linear model depends on the variability of the spectral data. The spectral variations characteristic of a given constituent are often weak and scattered on several principal components. It is therefore necessary to take the chemical data into account to get spectral patterns. For this, multiple linear regressions are assessed with the principal-component scores as independent variables (principal-component regression, PCR). The number of terms in the equations is determined by the statistical Fisher F (Foucart and Lafaye, 1983). The predictions of the chemical data correspond to

$$\mathbf{y} = \mathbf{C}\cdot\mathbf{r} + \mathbf{1}\cdot r_0 \quad (5)$$

$\mathbf{y}$ = column vector of the predicted chemical data, $\mathbf{r}$ = column vector of the regression coefficients, r_0 = intercept, and $\mathbf{1}$ = column vector whose every element is unity. The spectral patterns are calculated from a combination of the eq 2 and 5:

$$\mathbf{y} = \mathbf{X}\cdot\mathbf{V}\cdot\mathbf{r} + \mathbf{1}\cdot r_0 \quad (6)$$

The projection of the original spectra on to the vector $(\mathbf{V}\cdot\mathbf{r})$ gives the predicted chemical data to within about r_0. The vector $(\mathbf{V}\cdot\mathbf{r})$ is called the spectral pattern.

RESULTS AND DISCUSSION

Chemical Analyses. The percentages of degradation of the dry matter were in the range 0–90%. The mean, standard deviation, and range values for the protein and galacturonic acid contents of the residues were shown in Table I. The protein content was negatively correlated to the galacturonic acid content with a coefficient R = −0.85.

Infrared Spectra. The normalized spectra of the nylon bag residues are shown on Figure 1. The absorption band at 1740 cm^{-1} could be assigned to the carboxyl groups of methylated galacturonic acids (C=O ester). The intensity of this band decreased with the incubation times. In the rumen, pectins were degraded and their ester functions disappeared. The amide II band that is characteristic of

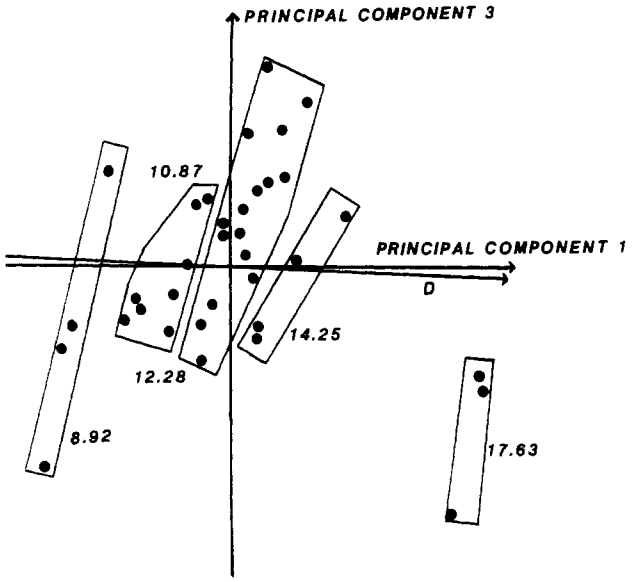

Figure 2. Similarity map of the spectra. Discrimination of the samples according to the protein content. The numbers are the mean protein contents of each group.

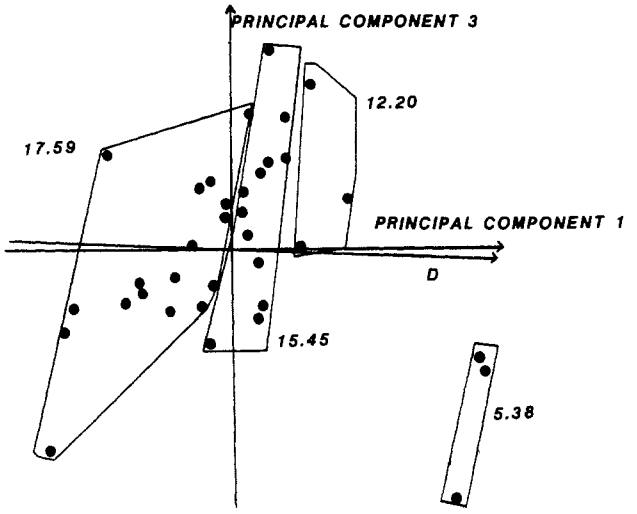

Figure 3. Similarity map of the spectra. Discrimination of the samples according to the galacturonic acid content. The numbers are the mean galacturonic acid contents of each group.

proteins was present in the area centered at 1530 cm^{-1}. In the nylon bag residues, the protein concentration increased during the rumen incubation. The intensity of the amide II band expressed this evolution of proteins. At 1645 cm^{-1} the amide I band overlapped the carboxyl band corresponding to the COO^- of pectins. The increase of protein content in the residues might be due to a poor digestibility. The organic matter and crude protein digestibilities of dried pulps are, respectively, 80% and 50% (Jarrige, 1978). However, the protein increase might be explained by microbial growth.

Similarity Map. The principal component 1 was correlated to the degradation of beet pulps (percentage of disappeared dry matter) with a coefficent $R = 0.91$. The major statistical information that was extracted by PCA was therefore the global degradation. Predictive equations could be established for protein and galacturonic acid contents by using PCR. These equations showed that principal components 1 and 3 were the more predictive variables. The similarity map defined by these principal components is given Figures 2 and 3. This map took 83.20% of the cumulated variance into account. The line

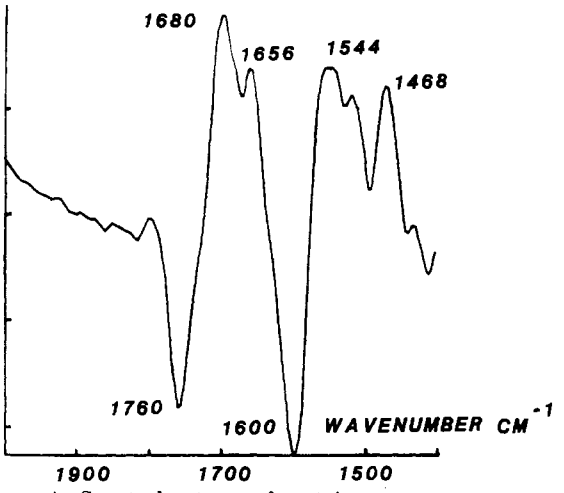

Figure 4. Spectral pattern of proteins.

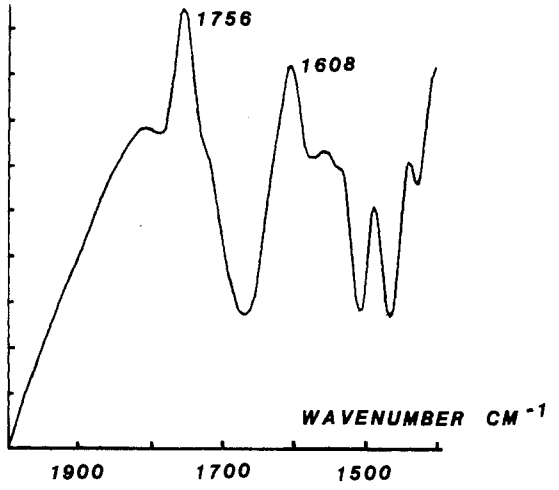

Figure 5. Spectral pattern of galacturonic acids.

D was the graph of the linear combination of principal components 1 and 3, which predicted the degradation with a coefficient of correlation equal to 0.92. On this similarity map, the spectra could also be classified according to their protein content (Figure 2) or their galacturonic acid content (Figure 3). These results showed that it was roughly equivalent to predict the protein and the galacturonic acid contents or the degradation, given that these parameters were strongly correlated.

Information about beet pulp degradation was obtained by applying PCA to the spectral data. The similarity maps could be used to assess the degree of degradation, the protein content, or the galacturonic acid content of an unknown sample (Robert et al., 1988). These evaluations would be obtained by projecting the spectrum of the unknown sample on to the similarity maps.

Spectral Patterns. The spectral patterns of proteins and galacturonic acids were calculated according to the equation (6) and are respectively given Figures 4 and 5. The spectral pattern of protein showed the amid II band at 1544 cm^{-1}, which was already visible by direct examination of the original spectra. The position of this band was accurately estimated and was in full agreement with the results of the literature (Zundel et al., 1984). The spectral area between 1700 and 1600 cm^{-1} was split by PCA into two peaks at 1680 and 1656 cm^{-1} that could be associated to the amide I band. The peak at 1468 cm^{-1} corresponded to methylene scissoring vibrations. This spectral pattern presented dips at 1760 and 1600 cm^{-1} that could be assigned to vibration bands characteristic of pectins. These minima expressed the negative correlation

between protein and pectin contents.

The spectral pattern of galacturonic acids revealed wavenumbers at 1756 cm^{-1} (C=O ester) and 1608 cm^{-1} (COO^-). PCA split the broad band of the original spectra at 1645 cm^{-1} into amide I bands and a peak at 1608 cm^{-1} that was assigned to the galacturonic acids.

CONCLUSION

The similarity maps and the spectral patterns obtained by PCA give complementary information on the collection of spectra being studied. The maps enable spectra to be easily compared with each other. When the contents of different biochemical data are correlated, the observation of the similarity maps provided a global interpretation of the spectra. The directions in the PCA space characteristic of a given phenomenon are obtained by PCR. A representative spectral pattern can be associated by the described procedure to each direction so created. The spectral patterns reveal the spectral information that is significant about a particular prediction.

Middle-infrared spectroscopy is applicable to the study of the microbial degradation of natural products rich in cell wall components. Qualitative information may be obtained with the procedures presented. Previous studies (Isaksson and Naes, 1987) showed that PCR allows quantitative applications.

Registry No. Galacturonic acid, 685-73-4.

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Influence of Alcohol-Containing Spreading Solvents on the Secondary Structure of Proteins: A Circular Dichroism Investigation

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The conformational properties of a variety of proteins have been investigated in solution in the presence and absence of a propan-2-ol-based spreading solvent by circular dichroism spectroscopy. β-Casein, ovalbumin, α-lactalbumin, and β-lactoglobulin all exhibited large conformational changes consistent with the induction of α-helix into their structure. The influence of type and concentration of alcohol and temperature on the conformational properties of the "classical random protein", β-casein, has been investigated in detail. The effect of alcohol has been shown to be reversible upon dilution with biphasic kinetics. The slow rate of the second process indicates that in spreading experiments the protein is initially present at the interface in an altered conformation compared to that of adsorbed protein. This may have important consequences for the intermediate and final surface denatured state of the protein.

The physical measurement of interfacial properties of a wide variety of proteins has received considerable attention over the past few years. The principle aim of this effort has been to gain insight into the mechanism of protein stabilization of interfaces in foams and emulsions.

The most widely used approach adopted in this field has involved the study of macroscopic interfaces on Langmuir troughs. With use of this technique the formation of a protein layer at the air/water interface is possible by either of two methods, specifically by adsorption to the surface from the subphase or by spreading a protein monolayer directly onto the surface. It is the consequences of the latter technique that will be addressed in this study.

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Quantitative spreading of proteins has been most readily achieved by introducing the protein dissolved in an organic/aqueous solvent mixture as spreading solvent, where the protein is introduced to the interface with a microsyringe. Spreading from an aqueous solution is more difficult. In this case the protein solution is introduced to the surface by passing it down a perfectly wetted glass rod (Trurnit, 1960) at a moderate pH near the isoelectric point of the protein. These conditions reduce the energy barriers to adsorption.

A 60:40 propan-2-ol–water solution was first used for spreading serum albumin by Stallberg and Teorell (1939). Other workers have used the same spreading solvent with the addition of small quantities of isoamyl alcohol or sodium acetate (MacRitchie and Alexander, 1963; Harrap, 1955; Dervichian, 1939) to spread proteins such as BSA, insulin, and ovalbumin, respectively. More recently milk proteins have been spread with a 66% propan-2-ol solution

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