Tenderization of Beef with Pineapple Juice Monitored by Fourier Transform Infrared Spectroscopy and Chemometric Analysis

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ABSTRACT

For nondestructive monitoring of meat tenderizing processes, factor analysis, and evolving factor analysis (EFA) were applied to successively collected attenuated total reflectance-infrared spectra of beef after treatment with pineapple juice. Absorption around 1600 to 1500 cm^{-1} and 1400 cm^{-1} regions increased with time. After subtracting the starting spectrum from each of the succeeding spectra, factor analysis and EFA were applied to the resulting data matrix. Factor scores showed a time dependent change, but a clear difference between treated and reference beef was observed in factor 1. Eigenvalues calculated from EFA showed the existence of two vigorous digestion periods during tenderization, which supported the results from biochemical analysis.

Key Words: beef, bromelain, factor analysis, EFA, infrared

INTRODUCTION

SOME PROTEOLYTIC ENZYMES FROM plants or animals have been widely used as meat tenderizers for food processing in home cooking and industrial treatment. Pineapple or a pineapple protease, bromelain, is one of the most commonly used. Comparing bromelain with papain and ficin, characteristics of bromelain are not well understood. As meat tenderizers, research on working mechanisms of bromelain has mainly been conducted using conventional biochemical or chromatographic methods (Kang and Rice, 1970; Du Bois et al., 1972; Fogle et al., 1982; Goodenough and Owen, 1986; Dransfield, 1994). Most research on the digestion mechanism of meat proteins has been carried out using homogenized meat and sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis of soluble proteins. Brooks et al. (1985) investigated the changes of chicken muscle caused by crude papain treatment using densitometric tracing on SDS gels. According to SDS-PAGE profiles, which showed degraded fragments of meat proteins, progress in digestion was clearly observed on both myosin heavy chain (MHC) and actin by papain and bromelain into lower molecular fragments with time (Kim and Taub, 1991).

Most spectrometric methods, e.g., ultraviolet (UV), mid-infrared (IR), near-IR, Raman, and nuclear magnetic resonance, are nondestructive and can be used to monitor time-dependent structural changes of meat proteins (Örsi and Major, 1973; Chen and Lord, 1976). Some studies have described results of monitoring ongoing chemical reactions using IR analysis (Yamamoto and Tasumi, 1988; Bayada et al., 1995; Heberle and Zscherp, 1996). Further, mechanisms of reactions taking place could be monitored because relationships between absorption frequencies and chemical structures were well understood in IR spectrometry. In this context, attenuated total reflectance (ATR)-IR measurements were successfully applied in investigating the hydrolysis of trimethyl phosphate in solution (Bayada et al., 1995) and in monitoring conformational changes of bovine ribonuclease A induced by thermal treatment (Yamamoto and Tasumi, 1988). Our objective was to apply ATR-IR for continuous monitoring of structural changes in meat proteins caused by protease treatment. However, chemical changes in protein structures caused by meat tenderizer should be complicated and signals from such changes were expected to be weak.

Chemometric pattern recognition techniques are versatile for extracting useful information on chemical structures from complicated spectral data (Martens and Næs, 1989; Brown, 1995). Principal component analysis and factor analysis can easily separate signals relating to chemical structures from noise contained in any spectral data matrix (Robert et al., 1987). Another objective was to apply factor analysis to successively collected IR spectra for isolating information on structural changes in beef proteins. Evolving factor analysis (EFA) was applied to the spectral data matrices for extracting changes in variances relating to progress of tenderization with time. EFA could effectively detect uncorrelating and time dependent information in the data matrix by increasing row vectors one by one (Maeder, 1987; Keller and Massart, 1992).

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MATERIALS & METHODS

Sample beef and pineapple juice treatment

For IR analysis, frozen lean thigh strips of beef (5 cm × 1 cm × 0.5 cm) were thawed in a refrigerator until the surface temperature reached 5 °C measured by a digital surface thermometer HLC-60 (Anritsu Meter Co., Ltd., Tokyo, Japan). Then, one side of the beef strip was soaked in 1 mL of pineapple juice at 5 °C for 10 min to cause sufficient penetration of the pineapple juice into the inside of the beef and assure that it remained there after removing excess solution. Prior to soaking the beef strip in pineapple juice, the activity of bromelain was adjusted to about 200 units/mL by adding 50 mM phosphate buffer (pH 5.0) to the pineapple juice. Excess juice was removed from the beef strip with filter paper before spectrophotometric analysis was begun. The reference beef was treated in the same way, except boiled pineapple juice was used. The beef treated with pineapple juice and the reference beef are referred as B-beef and R-beef, respectively.

Spectral measurements

IR spectra were obtained from 4000 to 700 cm^{-1} at 20 °C, using a BIO-RAD model FTS45RD fourier transform (FT)-IR spectrophotometer, equipped with a ZnSe crystal ATR accessory (Cambridge, Mass., U.S.A.). Absorbances at 65 wave numbers (cm^{-1}) or frequencies in the 1800 to 1300 cm^{-1} region were selected with equal intervals for multivariate data analysis. After the temperature of beef reached 20 °C within 2 min, each IR spectrum was successively collected at every minute up to 20 min. The acquisition time, 0 to 20 min, was decided from preliminary observations. Water signals were manually subtracted from every IR spectrum.

Data analysis

Prior to factor analysis and EFA, in both B-beef and R-beef spectra, the IR spectrum at 0 min was subtracted from each

of the subsequent spectra using EXCEL 97 (Microsoft Corp., Seattle, Wash., U.S.A.). Factor analysis and EFA were performed by SYSTAT ver. 5.0 (SYSTAT, Evanston, Ill., U.S.A.) on the basis of the covariance matrix.

Factor analysis

Factor analysis is a widely used technique in chemometrics (Dillon and Goldstein, 1984). The purpose of factor analysis is to extract the latent factor(s) existing in a complex data matrix. Samples have been classified more clearly by extracted factors than by raw spectral data (Bertrand et al., 1985). Original spectra for individual components were identified from spectra of mixtures using factor analysis (Robert et al., 1987). When a data matrix, $\boldsymbol{X}$, composed of q IR spectra measured at s frequencies, q and s being 20 and 65, respectively, in this research, the basic model for factor analysis was $X = Vf + e$ with algebraic representation as follows:

$$x_1 = v_{11} f_1 + v_{12} f_2 + \ldots\ldots + v_{1q} f_q + e_1$$

$$x_2 = v_{21} f_1 + v_{22} f_2 + \ldots\ldots + v_{2q} f_q + e_2$$

$$x_s = v_{s1} f_s + v_{s2} f_2 + \ldots\ldots + v_{sq} f_q + e_s$$

Here, there are q ($<s$) common factors denoted by f_j, j = 1, 2,---, q , where v_{ij} , i = 1, 2, ---, s and j = *1, 2*,----, q, give the weights for jth common factor (factor loading), and e_i gives s unique factors or errors. In this model, the eigenvalue of jth factor means the variance in the jth factor extracted from data matrix, $\boldsymbol{X}$. By applying the factor analysis to a data matrix, $\boldsymbol{X}$, common factor(s), f_j, can be separated from error, e_i. Extracted common factors were expected to produce less noisy results from calibration or pattern recognition analysis.

Evolving factor analysis (EFA)

The data matrix, $\boldsymbol{X}$, was composed of 20 rows or samples and 65 columns or frequencies in the IR spectra. Factor analysis has been widely used to search for common structures underlying a complicated data matrix. However, EFA has been used to determine equilibrium situations existing mainly in high performance liquid chromatographic (HPLC) data recorded using a photodiode array detector (PDAD), since the first appearance of this methodology (Maeder 1987).

The purpose of EFA (Fig. 1) is to follow the changes in eigenvalue of the $\boldsymbol{X}$ matrix obtained from continuous observation of changes progressing with time by eigenvalue analysis of the submatrices $\boldsymbol{Xi}$ formed by the first *1,2, ..., i,...*, s rows in the $\boldsymbol{X}$ matrix. First, submatrices from which a factor(s) is extracted must be defined. Data sets are successively created by adding a row, one by one to the preceding matrix until whole data are treated by EFA. In EFA, eigenvalues or uncorrelating variances contained in each submatrix are calculated by factor analysis. In the forward EFA, factor analysis is started from the first submatrix toward the last submatrix or whole data matrix. In this way, if a change would start to occur at a certain time, the corresponding eigenvalue of the first factor would appear and, then, increase with the number of rows. If another change would occur afterwards, it could be detected as a new eigenvalue corresponding to the second factor. Thus, the number of different changes could be detected as the number of factors. Conversely, in the backward EFA, factor analysis is successively performed from the opposite direction, starting from the last row and toward the first row by increasing the number of rows one by one. We applied this approach to the data matrix composed of successively collected IR spectra, which were expected to contain information on the digestion process of meat proteins with bromelain. If only a single digestive reaction were to occur during the tenderizing process, then only one factor would be extracted. However, if the meat tenderizing process were to evolve through 2 or more different reactions starting one after another, the corresponding number of factors would be extracted.

Thus, in EFA, the eigenvalue changes depend solely on observed measurements that can be classified into type I and type II on the basis of their occurring modes. Assuming a fully separated peak recorded by HPLC-PDAD as an example for type I, we can observe that absorption starts to increase from the baseline level and eventually returns to the initial level after reaching an apex. On the other hand in type II, like the meat digestion process, we assume that a substance A would be transformed into a product B without any reverse reaction, where both A and B would be present to some extent. Thus, absorption of both A and B should not be zero from the beginning. In the type II, eigenvalues in EFA keep increasing in accordance with the number of rows because every row contains more or less variance. Eigenvalue changes classified into types I and II were compared (Fig. 2). For showing the eigenvalue change in the type II more clearly, it was useful to divide each eigenvalue by the corresponding number of rows, since influence from the increase in rows on eigenvalues could be compensated for by such averaging process. Therefore, we incorporated the averaging process of eigenvalues obtained from EFA.

RESULTS & DISCUSSION

IR spectra

All IR spectra were sorted according to time (Fig. 3). Two strong absorption bands observed at 1638 and 1549 cm^{-1} became even stronger with time in B-beef spectra and R-beef spectra. The absorptions around 1638 cm^{-1} and 1549 cm^{-1} were assigned to the amide I band and amide II band, respectively (Bellamy, 1958). Further, a small peak, observed around 1400 cm^{-1} and ascribable to carboxylic anion ($-COO^-$), was distinctive in both IR spectra.

Factor analysis for IR spectra of B-beef and R-beef

After combining B-beef spectra and R-beef spectra, factor analysis was applied to the merged matrix. The proportion of variances explained by factor 1 and factor 2 were 95.1% and 3.4%, respectively. However, significant difference in score patterns of factor 1 between the 2 beef samples indicated that information on digestion of beef proteins was mainly extracted in factor 1 (Fig. 4). Further, the movement of factor 1 scores in B-beef showed 2 step changes, one finished by 5 min and another from 12 to 15 min.

Heavy loadings on factor 1 of two re-

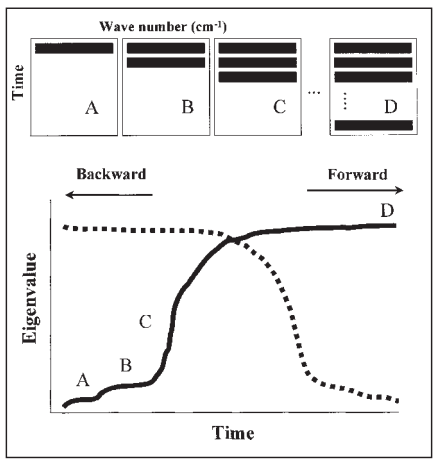

Fig. 1—Eigenvalues calculated from hypothetical data matrices (A to D) using forward EFA and backward EFA.

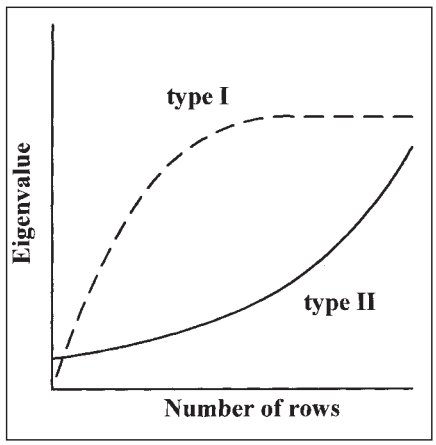

Fig. 2—Comparison of eigenvalues calculated from EFA applied to hypothetical data matrices expressing type I and type II phenomena.

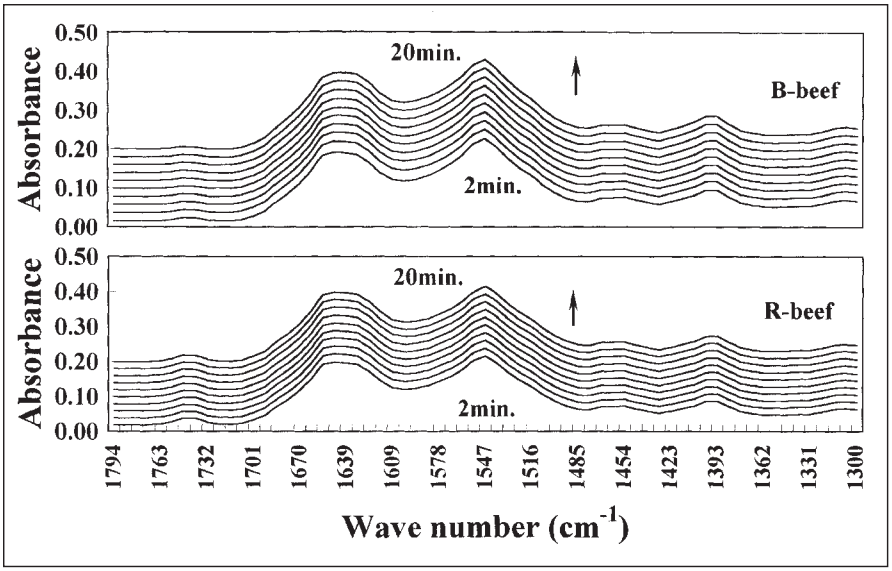

Fig. 3—IR spectra of B-beef and R-beef strips successively collected from 0 to 20 min.

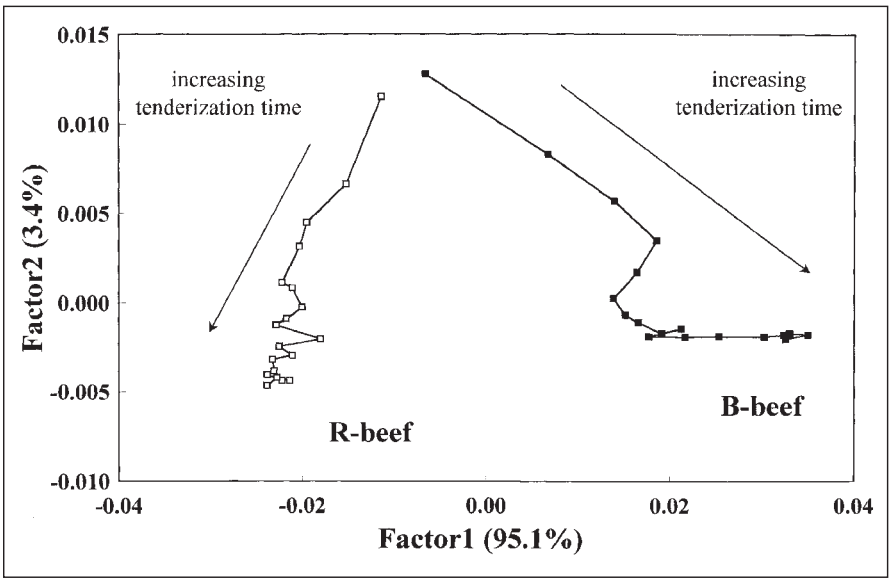

Fig. 4—Factor scores calculated from a data matrix combining B-beef spectra and R-beef spectra together.

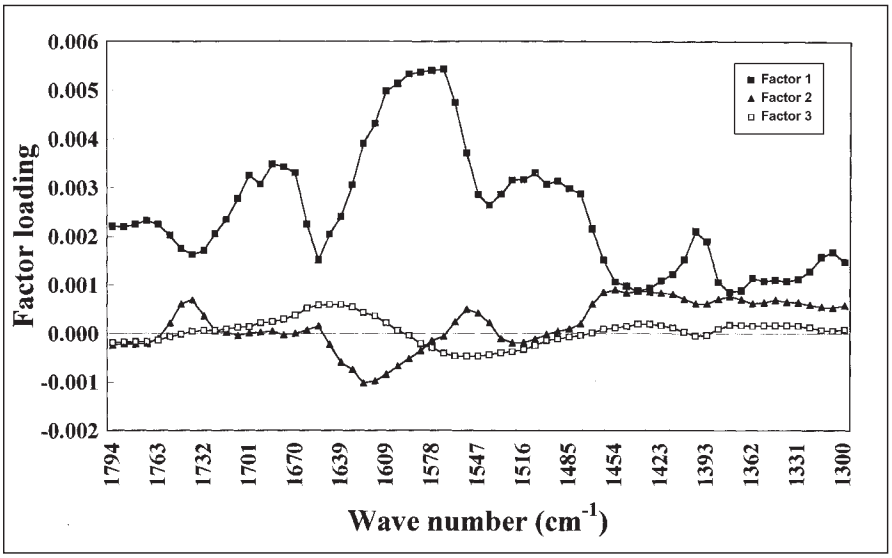

Fig. 5—Factor loadings of combined B-beef and R-beef spectra on factors 1-3

gions, around 1400 cm^{-1} ascribable to carboxylic anion and 1575 cm^{-1} ascribable to simple peptides (Bellamy, 1958), were indicated (Fig. 5). Further, 2 broad peaks around 1685 and 1510 cm^{-1} were observed in the loading pattern for factor 1. One peak around 1680 cm^{-1} seemed to closely relate with peptide bands but another could not be clearly assigned. Factor 1 seemed to reflect the digestion of high molecular beef proteins into lower molecular fragments because these high loading regions closely relate to N-terminal amino group or C-terminal carboxyl group. Therefore, increase in factor 1 scores in B-beef with time seemed to indicate time-dependent increase in peptides or amino acids in beef. However, factor 2 seemed to contain a certain time-dependent change but did not directly relate to the digestion of proteins because factor 2 scores calculated for both B-beef and R-beef similarly decreased with time. Compared to loading at 1610 cm^{-1}, loadings at 1739 and 1554 cm^{-1} regions on factor 2 were weaker. However, absorption around 1610 cm^{-1} could be simply ascribable to water molecules. Therefore, factor 2 seemed to indicate the time-dependent increase in amounts of released water from beef strips by its own weight.

Thus, by comparing factor score patterns calculated from B-beef spectra and R-beef spectra together, differences in time-dependent structural changes between B-beef and R-beef were clearly indicated.

EFA

Eigenvalues calculated from forward EFA did not show any change relating to time since forward EFA of B-beef could not detect the changes caused in beef because of large variances in the first 5 min. However, eigenvalues for R-beef did not show any significant change in 20 min. The backward EFA of B-beef seemed to succeed in tracing back the structural changes in beef proteins, while that of R-beef did not show any significant changes (Fig. 6). Two distinctive changing periods, i.e., 2 to 5 min and 12 to 15 min, observed in the backward EFA of B-beef suggested that the beef digestion with bromelain progressed through 2 steps. As clearly shown by backward EFA of B-beef, the digestion of beef proteins by bromelain was so rapid in the initial 5 min that the forward EFA failed in tracing such structural changes. In contrast, the backward EFA succeeded in extracting the time dependent change because of the stationary condition existing in B-beef after 15 min, since vigorous digestion of proteins might have almost finished by 20 min.

Conventional factor analysis was separately applied to each of 4 submatrices composed of IR spectra in 2 to 5, 6 to 11, 12 to 15, and 16 to 20 min periods of B-beef, and the resulting factor loading patterns of 4 periods were compared (Fig. 7). Two loading

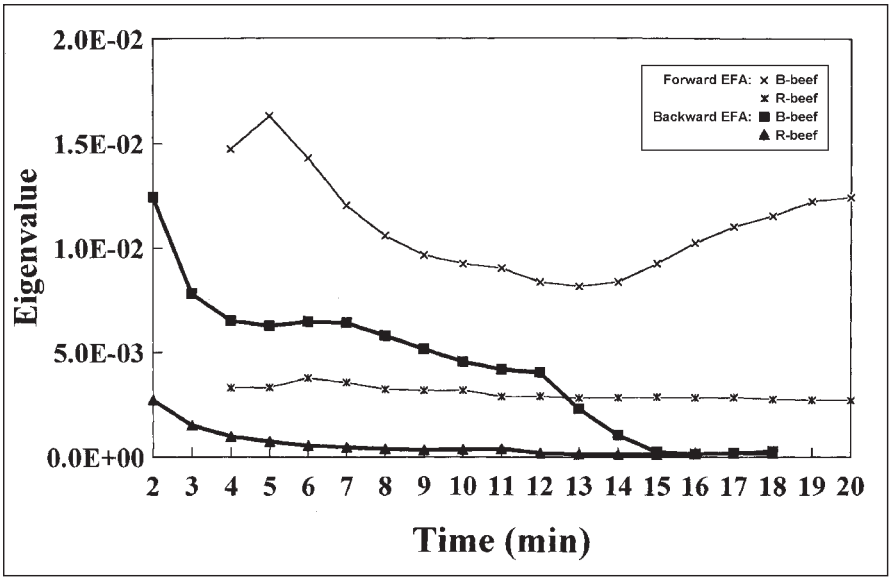

Fig. 6—Eigenvalues of factor 1 calculated from IR spectra of B-beef and R-beef using EFA.

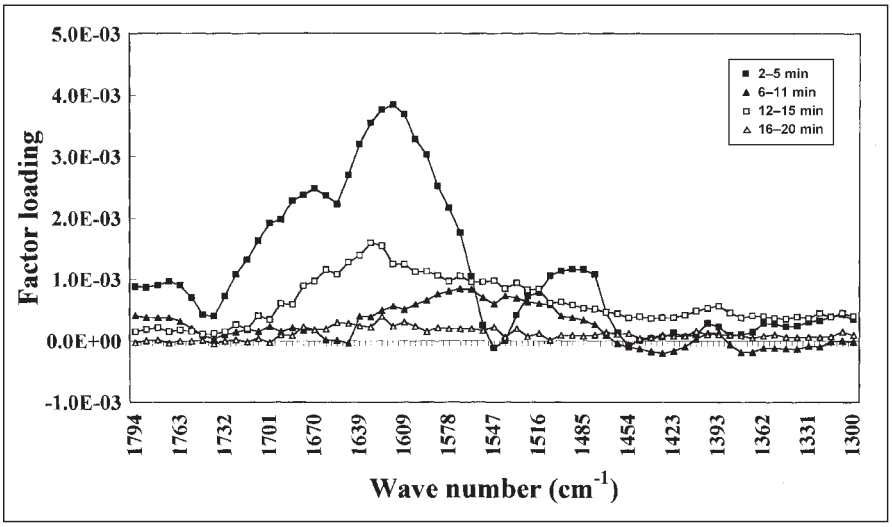

Fig. 7—Factor loadings patterns of IR spectra of B-beef on factor 1 calculated for 4 time periods

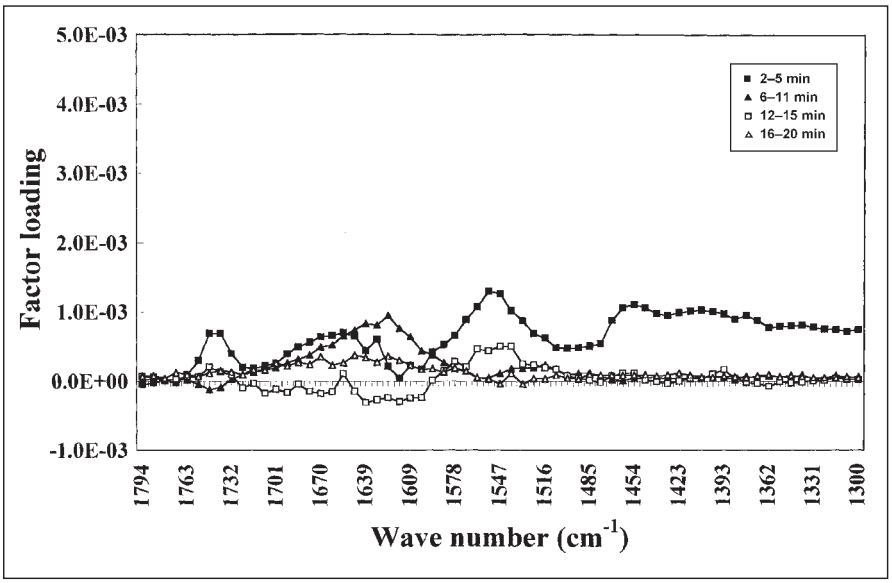

Fig. 8—Factor loading patterns of IR spectra of R-beef on factor 1 calculated for four time periods.

patterns in 2 to 5 min and in 12 to 15 min were not exactly the same, but they were similar in that both commonly indicated the increase in absorption associated with the > N-H stretching band. Thus, heavy loadings of amide related areas on factor 1 seemed to reflect the rapid increase in eigenvalues in the 2 periods. However, factor loadings of IR spectra in both 6 to 11 min and 16 to 20 min periods did not show any clear patterns related to chemical structures in beef proteins and supported the smaller changes in eigenvalues in the 2 periods.

Eigenvalues calculated from backward EFA applied to R-beef spectral matrix did not show any apparent change relating to time (Fig. 6). Factor loadings calculated for 4 periods — 2 to 5, 6 to 11, 12 to 15, and 16 to 20 min — of R-beef spectra did not correlate with specific chemical structures (Fig. 8). Further, these factor loading patterns were different from those calculated for B-beef spectra. Thus, the time-dependent changes extracted from IR spectra of B-beef could be ascribable to the digestion process of muscle proteins caused by bromelain treatment.

DISCUSSION

APPLICATIONS OF FT-IR/ATR SPECTROMETRY to food analysis have potential advantages in several aspects. Progress in data handling techniques aided by advance in chemometric methodologies and computer technology have made it possible to solve problems caused mainly due to abundant noise in spectroscopic data. Our success in extracting significant information on meat digestion from IR spectra was based on the applicability of factor analysis and EFA in handling complicated data matrix. Particularly, EFA seems useful for depicting time-dependent change of beef protein, since this deconvolution method could clearly indicate the existence of two vigorous digestion periods as rapid changes in eigenvalues. The two-step digestion process of beef proteins has been reported by Kim and Taub (1991). They observed preferential digestion of myosin heavy chain and subsequent actin digestion by bromelain, using SDS-PAGE of solubilized proteins. Comparing such biochemical methods, the spectrometric method followed by chemometric data analysis facilitated the monitoring of meat tenderizing.

Another advantage of the spectrometric method is the possibility of directly discerning the digestion mechanism of meat proteins on the basis of factor loading patterns of each time period. These patterns can provide useful information on changes in chemical structures.

By including an ATR accessory used in this types of research, applications of FT-IR spectrometry have rapidly progressed into analyses for various materials, including foods. Spectrophotometers that can simultaneously obtain FT-IR and near-infrared

(NIR) spectra and are equipped with an ATR accessory have been developed. The accuracy and nature of information about meat digestion process should be further improved by using such an advanced instrument; spectra in the NIR and IR regions could be successively obtained from the same part of the same sample under the same conditions (Thomas, 1994).

It is essential to monitor textural changes in the tenderizing process of meat to avoid producing excessively tenderized products. However, it is difficult to evaluate meat tenderness objectively and nondestructively. Thus, it is further evaluated based on sensory tests by individuals. Although analysis relating to texture was not conducted, our results suggested that the degree of meat tenderness may be predicted using the spectroscopic approach, which combines information from texture measurements and/or sensory evaluation.

CONCLUSIONS

ALTHOUGH VISUAL COMPARISON OF IR spectra from B-beef and from R-beef did not indicate any apparent differences, factor analysis and EFA applied to spectral matrices showed significant differences. Eigenvalues extracted by EFA were effective for detecting and depicting time-dependent changes. In both factor analysis and EFA, factor loading plots clearly indicated structural information in the extracted factors. An approach combining spectroscopic observation and subsequent multivariate data analysis could be applied not only to meat science and technology but to other types of food. Advantages of these spectroscopic analyses lie in their nondestructive nature and capability for continuous sample measurements.

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