

Starch Gelation Process Observed by FT-IR/ATR Spectrometry with Multivariate Data Analysis

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ABSTRACT

For directly observing changes related to the gelation process of starch, IR spectra of starch in water while heating were obtained using FT-IR/ATR spectrometry. Relationships between gelation and spectral changes were examined using factor analysis, evolving factor analysis (EFA) and three-way principal component analysis (PCA). Absorption at 3300 and 1610 cm^{-1} decreased with temperature but absorption at 1000 cm^{-1} increased. The factor score plot patterns of amylose, amylopectin and rice starches were similar but those of potato and corn starches were unique. EFA indicated variances relating to changes caused in starch and water as different factors. Loadings of the starch component 2 in three-way PCA correlated with starch granule sizes.

Key Words: starch, gelation, FT-IR/ATR, EFA, 3-way PCA

INTRODUCTION

GELATION OF STARCH MUST BE CAREFULLY controlled because the desirable viscosity of the final product is critical for consumers preference. Viscosity is influenced by amylose and amylopectin contents (Miles et al., 1985; Ring et al., 1987). The iodine binding capacity of starch is widely used for measuring amylose content. Many analytical methods could also be applied such as differential scanning calorimetry (DSC) (Stevens and Elton, 1971; Kim et al., 1995; Mestres et al., 1996), visible spectra (Jarvis and Walker, 1993) or rapid visco analyzer (RVA) (Haase et al., 1995). Campbell et al. (1996) and Rao et al. (1997) found relationships between rheological properties and granule sizes of starches. NMR (Seow and Teo, 1996; Nilsson et al., 1996; de Graaf et al., 1995) and X-ray diffraction (Waigh et al., 1997) have been effective in elucidating starch structures, and microscope or scanning electron micrographs (SEM) can directly observe starch granules (Kawakami, 1975; Seguchi, 1995). Kawakami (1975) microscopically observed that raw starch granules swelled by trapping water with increasing temperature and finally dispersed.

Haase et al. (1995) reported that starch gel flowed out from granules after collapsing and viscosity increased. Wilson et al. (1991) indicated that DSC measurement was invasive and the order-disorder transition could not be surveyed due to its macroscopic nature. Infrared spectroscopic analysis is nondestructive and allowed direct observa-

tion of specific functional molecular groups during gelation. Regarding changes in starch polymers during gelation, they indicated that similar results were obtained from IR spectra and DSC curves but IR supplied more detailed information on conformational changes.

Utility of near-infrared spectroscopy (NIR) for quantitative and qualitative analysis of starch characteristics has been reported (Villareal et al., 1994; Delwiche et al., 1996; Zeng et al., 1996). Due to the large absorption caused by water molecules, the infrared region had generally been considered inappropriate for food analysis. However, the great improvement in the S/N ratio in Fourier transform infrared (FT-IR) spectrometry has made it possible to utilize it for food analysis (Chang et al., 1995; McKelvy et al., 1998). FT-IR analysis has been used to successfully examine starch gelation (Wilson et al., 1987; Wilson and Belton, 1988), determine individual sugar contents during starch hydrolysis (Bellon-Maurel et al., 1995) and screen infected potato tubers (Stewart et al., 1994). In starch retrogradation, Wilson et al. (1991) showed that the sensitivity of IR measurement was adequate and similar to that of DSC.

One of the advantages of spectrometry lies in non-destructive and successive sample measurements during food processing, i.e., heating or cooling (Yamamoto and Tasumi, 1988; Mizoguchi, 1994). Attenuated total reflectance (ATR) IR spectrometry using a water resistant ZnSe or Ge crystal can be applied to samples unsuited for transmittance studies. Our objective was to apply an FT-IR/ATR system equipped with a heating accessory to continuously observe the same

starch sample for chemical changes during gelation.

Progress in data analyzing aided by improvements in computer technology enables extraction of useful information from complicated and noisy spectral data (Meglen, 1988; Lavine, 1998). Chemometrics techniques have been applied to NIR spectra (Mark, 1989; Gemperline and Webber, 1989; McKelvy et al., 1998) which have been successfully analyzed by pattern recognition (Millar et al., 1996; Windham et al., 1997). Evolving factor analysis (EFA) has been applied to mainly deconvoluting three-dimensional data from HPLC analysis (Maeder, 1987; Keller and Massart, 1992). In FT-IR spectra, EFA was applied to monitoring a meat tenderizing process (Iizuka and Aishima, 1999) and molecular transformations in acid-base reactions (Esteves da Silva et al., 1996). Three-way principal component analysis (PCA) has mainly been used for problems in social sciences (Kroonenberg, 1983) but several successful applications to chemical problems have been reported (Geladi, 1989; Zeng and Hopke, 1990; Henrion et al., 1995). Three-way PCA can analyze a data matrix composed of three changeable variables, such as wave numbers (cm^{-1}), heating temperature ($^{\circ}\text{C}$) and types of starches in the FT-IR data from starch gelation.

MATERIALS & METHODS

Starch

Corn starch and potato starch were purchased from Wako Pure Chemicals Industries Ltd. (Osaka, Japan). Rice starch, amylose and amylopectin were reagent grade (Sigma, St. Louis, MO, USA). Distilled-deionized water was used to make all starch-water mixtures.

DSC and viscogram

Differential scanning calorimetry measurements were performed using a DSC 120 instrument (Seiko Instruments Inc., Tokyo, Japan). About 25 mg starch was placed on a 70 μL Ag sample pan, and water was added to make an equivalent weight mixture of starch and water. After hermetically sealing, the sample pan was heated from 20 $^{\circ}\text{C}$ to 90 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$. Water (50 mg) was used as reference. Viscograms were obtained using a rapid visco analyzer RVA-3D (Newport Sci-

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entific P/L, Warriewood, Australia). Starch (4 g) and water (24 g) were put in a sample tube. A paddle was inserted into the tube, and the tube was installed in the instrument. Paddle stirring speed was set at 960 rpm for the initial 10 sec and then decreased to 160 rpm. The sample temperature was kept at 30°C for the initial 1 min and then elevated from 30°C to 100°C at 2 °C/min.

ATR-IR

FT-IR spectra were measured by a MFT-2000 FT-IR microscope equipped with an ATR accessory utilizing a Ge crystal (JASCO Co. Ltd., Tokyo, Japan) (Fig. 1). After thoroughly mixing 1 g each of starch and water mixture in a vial, a part of the paste was placed on the surface of a Ge crystal using a spatula to make the paste cover the surface at uniform thickness. Then the Ge crystal was installed in the sample cell. The IR region measured was between 4600 and 650 cm⁻¹ with 4 cm⁻¹ resolution. Scans (100) were accumulated every 2 min, and IR spectra were stored in the JASCO-FT program. Spectra were consecutively obtained while a starch-water mixture was heated from 40°C to 94°C at 2°C/min using a programmable sample heater (Mettler-Toledo GmbH, Greifensee, Switzerland) (Fig. 1).

Data analysis

FT-IR data points (71), 3800-2800 cm⁻¹

at 100 cm⁻¹ increments and 1800-650 cm⁻¹ at 19 cm⁻¹ increments, were used for data analysis. Prior to factor analysis and three-way PCA, in every starch, IR spectrum at the starting temperature (40°C) was subtracted from each of the following spectra using EXCEL 97 (Microsoft Corp., Seattle, WA). Factor analysis and EFA were performed by SYSTAT ver. 5.0 (SPSS Inc., Chicago, IL) based on covariance matrices. The 3-WAYPACK (Section W.E.P., Leiden, Netherlands) was used for three-way PCA.

RESULTS & DISCUSSION

DSC and viscogram

DSC curves of corn starch and amylopectin showed large endothermic absorption at 66°C, but that of amylose at 52°C was much smaller (Fig. 2). The largest endothermic peak was found at 64°C for potato starch, but two small peaks appeared at 69°C and 80°C in the DSC curve for rice starch. Viscograms for five starches obtained by RVA were compared (Fig. 3). The viscogram for amylose showed little change, whereas a sharp peak appeared at 65°C in the viscogram for potato starch as had been indicated by Haase et al. (1995). The temperature for starting gelation was lowest in potato starch. The viscosity of rice starch reached a maximum at 90°C, the highest temperature for the maximum viscosity. Viscogram patterns suggested that gels formed by potato starch and amylopectin were fragile, while corn and rice starch gels were not easily broken up by the sheer rotation in RVA.

FT-IR spectra

IR spectral patterns of five starch powders were similar to each other (Fig. 4). Every spectrum showed four peaks at 3300, 1610, 1350 and 1000 cm⁻¹. The absorption regions at 3300 and 1610 cm⁻¹ were ascribable to water molecules, and that at

1350 cm⁻¹ to bending modes of O-C-H, C-C-H, and C-O-H angles (Bellon-Maurel et al., 1995). Absorption peaks due to water molecules were small in solid starch spectra. In the region between 1200 and 900 cm⁻¹, several strong absorption peaks assigned to C-C and C-O stretching modes appeared (Wilson and Belton, 1988; Bellon-Maurel et al., 1995).

IR spectra were obtained from starch-water mixtures during heating (Fig. 5). In every spectrum, absorption peaks at 1000 cm⁻¹ increased with temperature but spectral changes in amylose were much smaller than those in other starches. Wilson et al. (1991) compared the enthalpy calculated from DSC analysis with the absorbance ratio of two wave numbers (1047cm⁻¹/1150 cm⁻¹) in FT-IR spectra for the bread staling process and found similar trends. The absorption region at 1000 cm⁻¹ relates to C-C and C-O stretching modes of the polysaccharide backbone (Wilson and Belton, 1988; Bellon-Maurel et al., 1995) and increase in absorption in this region with temperature corresponded to the endothermic peaks in DSC curves (Fig. 2). In amylose, amylopectin and rice starch spectra, absorption of ranges at 3300 and 1610 cm⁻¹ showed little change with temperature. These two IR regions could

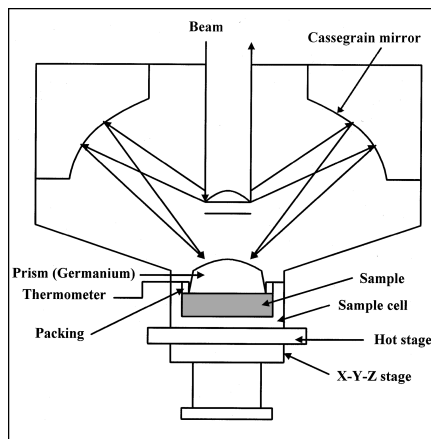


Fig. 1—Schematic of FT-IR microscope equipped with ATR accessory and programmable sample heater.

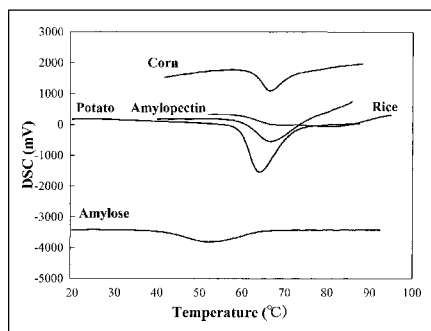


Fig. 2—DSC curves for five starches.

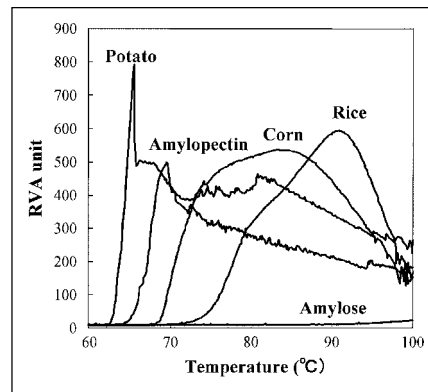


Fig. 3—Viscograms for five starches.

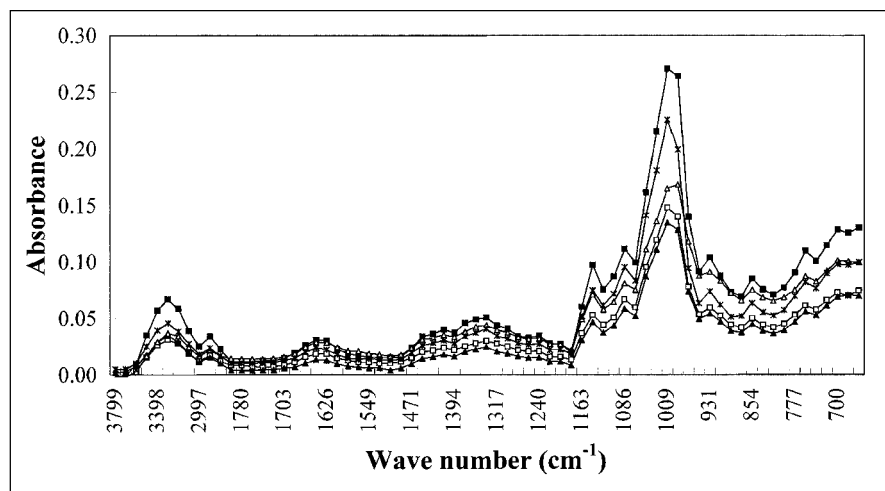


Fig. 4—IR spectra of five starches: ■ Amylose, □ Amylopectin, * Rice starch, ▲ Corn starch, △ Potato starch.

CHEMISTRY/BIOCHEMISTRY

be assigned to water molecules contacting the surface of Ge crystal. In amylose, amylopectin and rice starch, peaks appeared at 1000 cm^{-1} at certain temperatures, and their appearance seemed to closely relate with gelation (Wilson et al., 1987). During heating, temporal decreases in absorption at 3300 and

1610 cm^{-1} regions were observed only in the spectra of corn and potato starches. These decreases in absorption indicated a decrease in water molecules contacting the Ge crystal surface, and similar changes had been reported in the swelling process of starch granules using a microscope (Kawakami, 1975).

Factor analysis

As the result of factor analysis, factor 1 accumulated 79.8% of variances contained in the whole spectra, and factor 2 collected 17.5%. In amylose, amylopectin and rice starch, factor 1 scores increased with temperature, but increase in factor 2 was only slight (Fig. 6). Factor 1 scores of amylopectin and rice starch started to sharply increase at 66°C and 70°C , respectively, corresponding to the minimum points in their DSC curves (Fig. 2). Factor score patterns in amylose, amylopectin and rice starch were similar. Although score lines refracted at different temperatures, potato and corn starches were similar in factor score patterns. Factor 2 scores in potato starch started moving downward at 70°C and then returned after reaching the minimum at 80°C . Factor 2 scores in corn starch showed similar changes but reached the minimum at 78°C .

Factor loadings of wave numbers on factor 1 clearly indicated the close relationship with C–O and C–C vibrations in starches (Fig. 7). Comparing with DSC curves and viscograms, factor 1 scores seemed to express the starch gelation process by mainly focusing on changes in starch structure. Wave numbers loading heavily on factor 1 indicated an increase in contact of C–O and C–C portions on the Ge crystal surface with temperature. According to the factor loading pattern, the meaning implied in factor 2 could be interpreted as hydrogen bonding of water molecules contacting the surface of the Ge crystal. If so, factor score patterns of potato and corn starches might indicate temporal trapping of water in the interior domain of starch by loosening the rigid structure of granules and later release by complete rupturing of starch granules (Fig. 6). That is, the trapping and release of water by starch granules was reflected in the decrease and increase in factor 2 scores and such changes in factor scores seemed to be closely related to DSC and RVA observations. Thus, factor analysis facilitated in making complicated data more comprehensive by graphically illustrating the gelation properties of five starches but such comparison would be impossible if only FT-IR spectra had been compared.

EFA

The use of EFA enables following the change in the eigenvalue of the spectral matrix with time by eigenvalue analysis of the submatrices formed by the first 1, 2, ..., i , ..., s spectra. If some spectral change caused by a reaction is detected, the eigenvalue would start to increase. The first submatrix is a row composed of n columns corresponding to the wave numbers in IR spectra. In EFA, eigenvalues contained in each submatrix are calculated by factor analysis. If the second reaction would start, then it could be detected as the second eigenvalue, i.e., factor 2. Thus,

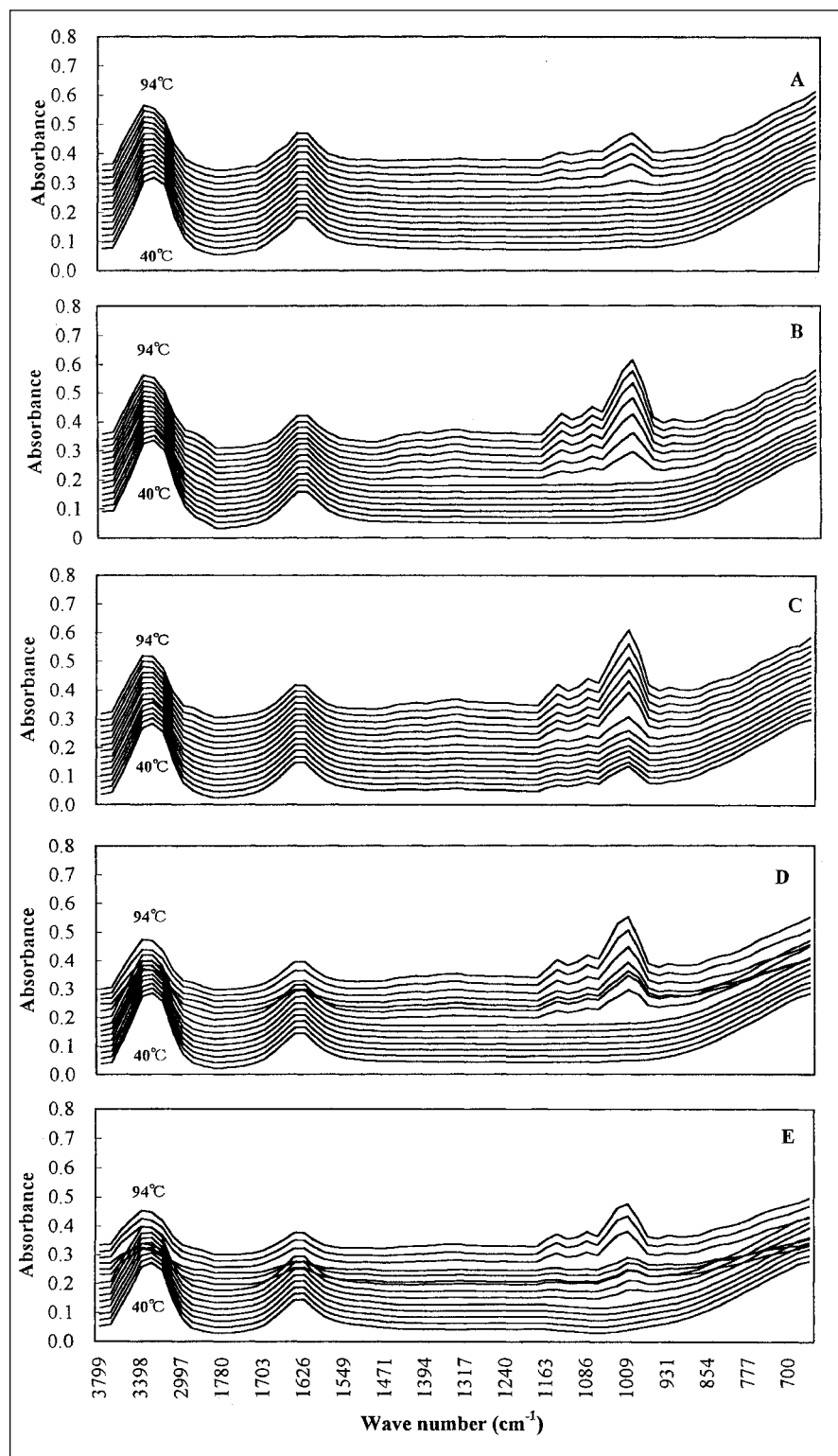


Fig. 5—IR spectra for aqueous mixtures of amylose (A), amylopectin (B), rice starch (C), corn starch (D), and potato starch (E) acquired with elevating temperature from 40 to 94°C at $2^\circ\text{C}/\text{min}$.

Table 1—Core matrix from three-way PCA of starch gelation

	Starch (G1)		Starch (G2)	
	Temp (H1)	Temp (H2)	Temp (H1)	Temp (H2)
Wave number (E1)	89.332	-5.112	-2.95	-6.019
Wave number (E2)	2.545	11.007	27.872	14.740

by plotting eigenvalues in EFA against time the starting point of a reaction(s) and its progress could be detected and the number of reactions could be found as the number of factors.

From EFA applied to corn starch spectra (Fig. 8A), eigenvalue plots for factors 1 and 2 indicated that the starch was gelated through two processes, the first one between 64°C and 94°C and the second between 74°C and 80°C. According to the loading plot (Fig. 7), a close relationship between factor 1 in EFA and the gelation process was expected. The factor loading plot also indicated that factor 2 corresponded to spectral changes between 74°C and 80°C and related to water content. Rapid changes in eigenvalues between 74°C and 80°C seemed to indicate trapping of water into granules due to the partial loosening of starch granules. However, as shown (Fig. 8B), rice starch spectra did not show any increase in eigenvalues of factor 2 in EFA. Thus, EFA could separate struc-

tural changes caused in the starch-water mixture during gelation process into individual factors.

Three-way PCA

Three-way PCA can indicate the variability in the three-dimensional data set, here composed of wave numbers (*i* in matrix **Z**), temperatures (*j* in **Z**) and starch types (*k* in **Z**) as shown (Fig. 9). The core matrix, **C**, explained the importance of combinations of **E**, **H** and **G**, each corresponding to the wave number components, temperature components and starch components, respectively. The largest three elements in the core matrix found in three-way PCA were 89.332, 27.872 and 14.740 as listed (Table 1). The combination of **E1-H1-G1**, each corresponding to wave number 1, temperature 1 and starch 1, respectively, was the largest element. The combinations for the remains two were **E2-H1-G2** and **E2-H2-G2**, respectively.

According to the loading plot for wave number components shown (Fig. 10A), **E1** correlated mainly with carbohydrate structure, since heavy loadings in the 1200–900 cm⁻¹ region were shown. However, **E2** closely correlated with water since two peaks at 3300 and 1610 cm⁻¹ were observed. The loading plots for **H1** and **H2** were considerably different from each other. Loadings for **H1** simply increased with temperature but those for **H2** showed a sharp increase starting at 72°C and a gradual decrease after peaking at 80°C (Fig. 10B). As indicated earlier, **E2-H2-G2** were closely associated with increase and decrease in the water molecules contact-

ing the surface of Ge crystal with temperature as **E2** (Fig. 10A) and **H2** (Fig.10B) indicated. The combination of **E2-H1-G2** highly related to water absorption at 3300 and 1610 cm⁻¹ and steady increase with temperature as **E2** and **H1** indicated. These trends were least in amylose and amylopectin but greatest in potato starch (Fig. 10C). As shown (Fig. 5), absorption peaks around 3300–3200 cm⁻¹ shifted from low to high wave number

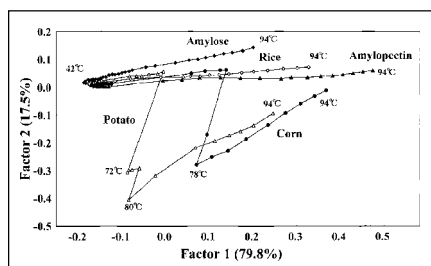


Fig. 6—Factor scores obtained from IR spectra of five starches and water mixtures.

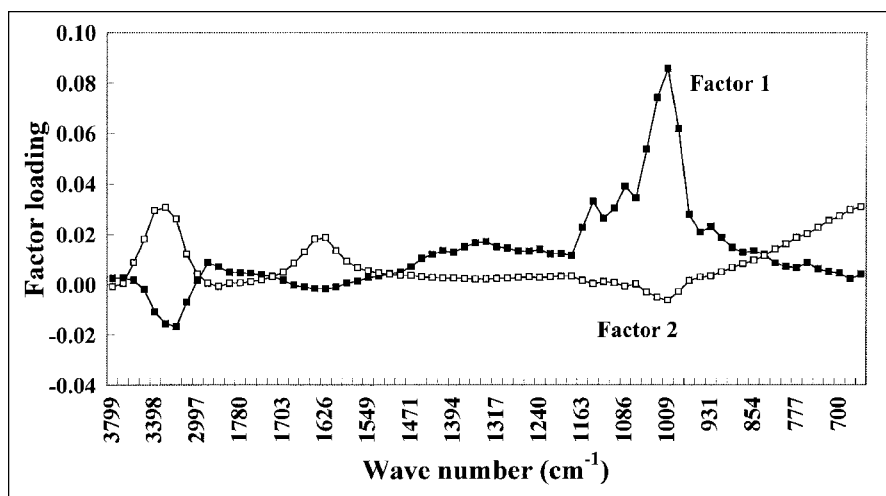


Fig. 7—Factor loadings of wave numbers of IR spectra on factors 1 and 2.

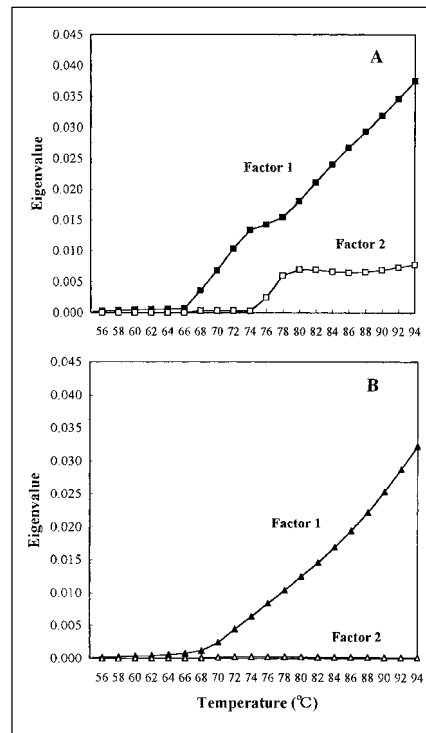


Fig.8—EFA plots obtained from IR spectra of corn starch (A) and rice starch (B).

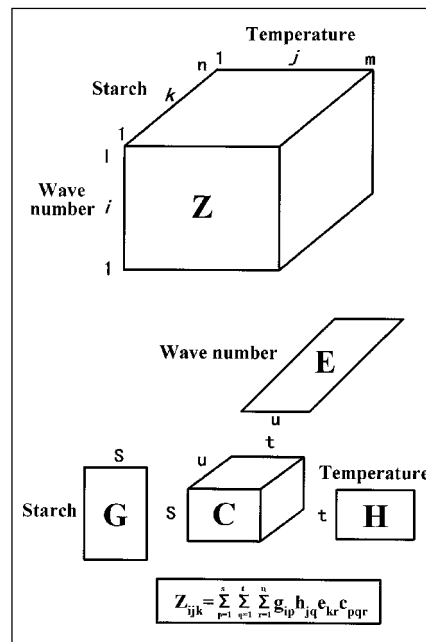


Fig. 9—Concept of three-way PCA.

region. This shift indicated that water molecules were trapped more rigidly by starch granules during gelation. Loadings at 3300 and 1600 cm^{-1} in E1 and E2 (Fig. 10A) gave different information on water molecules because negative loadings at 1600 cm^{-1} appeared only in E2 but negative loadings around 3300 cm^{-1} were found in both E1 and E2.

Thus, three-way PCA could separate information on the negative peak at 3200 cm^{-1} in E1 from that on the peak at 3300 cm^{-1} in E2. According to the loading plot for G1 and

G2 (Fig. 10C), 5 starches were classified into two groups based on G2 scores; one with amylose, amylopectin and rice starch, and another with corn and potato starches. After assigning amylose and amylopectin as granule size of zero since both of them have no granule structure, other starches were arranged according to granule sizes (Fig. 11). A linear relationship was found between loadings for G2 and their mean axis lengths calculated from 35 granules of each starch. Thus, a close relationship of the sizes of starch granules to gelation process was indicated by E2-H2-G2

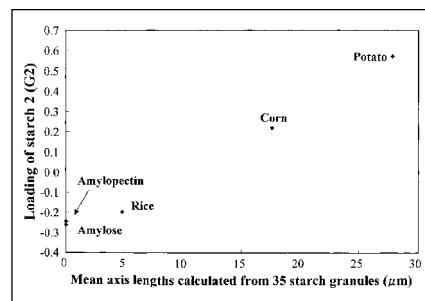


Fig. 11—Relationship between granule sizes of five starches and their loadings of starch 2 shown in Fig. 10.

in terms of granule swelling by trapping of water.

CONCLUSIONS

STARCH GELATION PROCESS WAS DIRECTLY observed by an FT-IR/ATR system equipped with programmable sample heater to perform real time and nondestructive monitoring of changes. Chemometrics approaches, such as factor analysis, EFA and three-way PCA, useful information relating to the gelation process from complicated IR spectra. Factor analysis and EFA supplied information on changes in water molecules and backbone structures in carbohydrates for monitoring gelation. Three-way PCA clearly revealed a relationship between granule sizes and trapping of water molecules in the gelation process. FT-IR/ATR spectrometry could add other information that could not be obtained from conventional analytical methods, e.g. DSC or RVA, by directly observing conformational changes. Thus, FT-IR/ATR spectrometry combined with appropriate chemometric techniques could become a useful methodology for food research and development because it can analyze samples containing abundant water. FT-IR/ATR may also successively accumulate spectra to trace changes in other major food components such as protein, lipid and mixtures during processing and storage. This requires further research.

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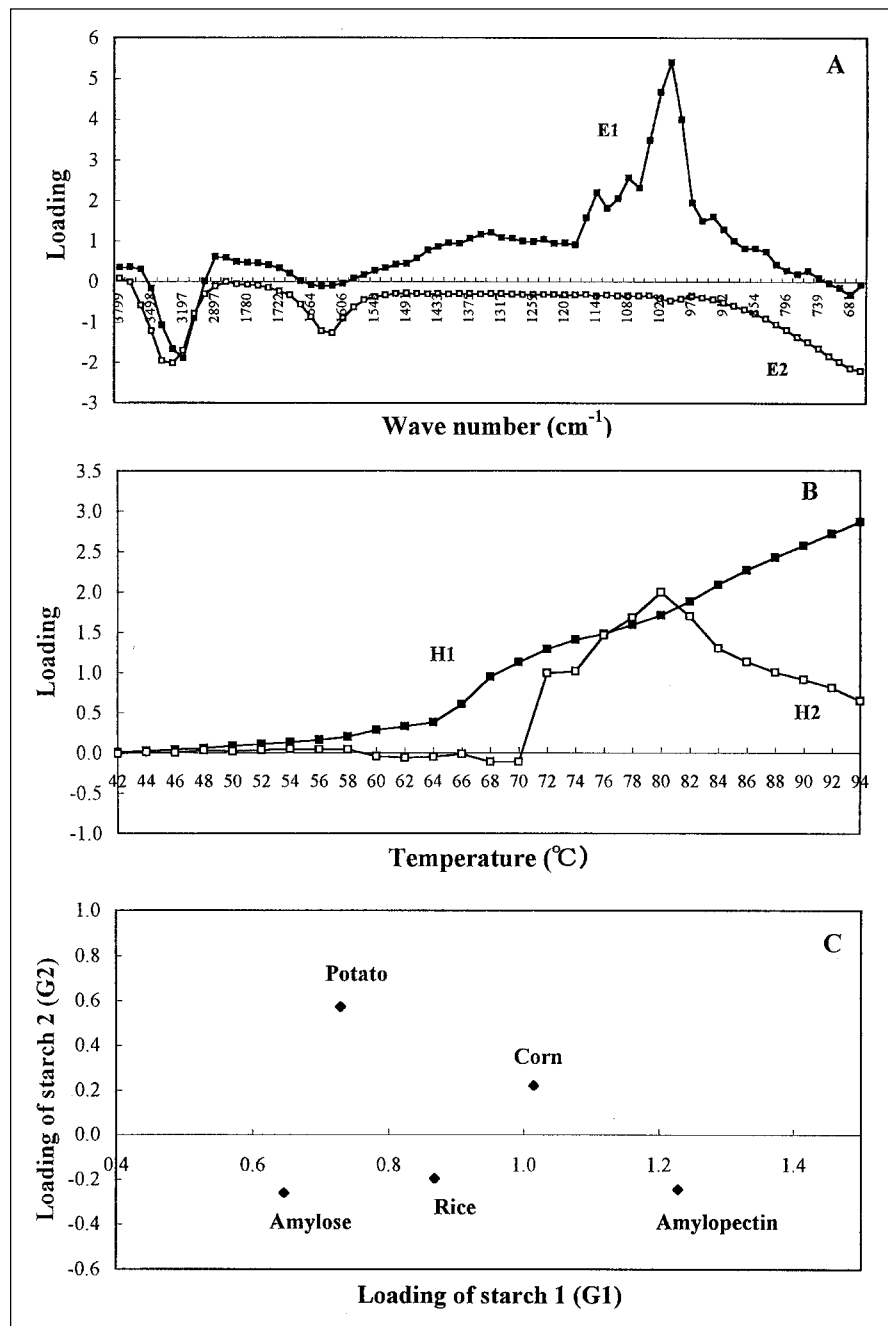


Fig. 10—Loadings for components 1 and 2 in wave number (A), temperature (B), and starch (C) in three-way PCA.

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