ARTICLES

Discrimination of Irradiated Starch Gels Using FT-Raman Spectroscopy and Chemometrics

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The effects of γ-irradiation on starch gels were characterized at the molecular level by Fourier transform (FT) Raman spectroscopy. Starches from five different sources were gelatinized and irradiated at 3, 5, and 10 kGy using a Co^{60} γ-irradiator. γ-irradiation effects on starch gels were noted by the C—H stretch ($2800-3000$ cm^{-1}) and O—H stretch ($3000-3600$ cm^{-1}) and bend ($1600-1800$ cm^{-1}) regions of the FT-Raman spectra. FT-Raman molecular fingerprints obtained through spectral analyses were used for discrimination of the gels based on the extent of irradiation by means of two different pattern-recognition techniques: canonical variate analysis (CVA) and soft modeling of class analogy (SIMCA). A complete discrimination of irradiated starches was attained using a hybrid partial least-squares (PLS) and CVA model, using the spectral variations in the C—H stretch and O—H stretch and bend regions of FT-Raman spectra. Using the same spectral regions, SIMCA predicted 84% of samples correctly.

INTRODUCTION

Gelatinization is one of the fundamental phenomena in starch-based foods. The basic mechanism of gelatinization can be described as the rearrangement of starch polymer networks in the presence of water through both inter- and intramolecular hydrogen bonding by means of a thermal process followed by controlled cooling.

Raman spectroscopy has long been used to monitor gelatinization and subsequent retrogradation of starch–water preparations (*1−3*). In these studies, gelatinization of different starches was screened by recording the changes in Raman "fingerprint" characteristics of granular starches particularly at 478, 1082, 1123, and 1340 cm^{-1}. Kim et al. (*2*) proposed a molecular-level mechanism for the gelatinization of maize starch based on the information obtained from Raman spectral responses recorded during the course of gelatinization. The involvement of water in the starch gelatinization process was well demonstrated by Schuster et al. (*3*) using a Fourier transform (FT) Raman approach. The authors reported that, whereas all of the polysaccharide-related Raman bands significantly decreased in intensity, only the bands associated with water uptake into the crystalline structure of starch (O—H bend and stretch) intensified or broadened upon gelatinization. Furthermore, Celedon and Aguilera (*4*) investigated the loss of birefringence in starch granules as a function of temperature by means of a Raman microprobe. The dominant Raman band due to the skeletal mode C—C stretch at 480 cm^{-1} was screened to determine the level of birefringence and monitor the advance of gelatinization with temperature.

Because of the ease in sampling, minimal interference by water, suppression of fluorescence, and sensitivity toward spatial conformational states of molecules, FT-Raman spectroscopy has been demonstrated as a promising tool to study food and biological systems (*5−7*). Both qualitative and quantitative analyses of spectroscopic data have been improved by applying advanced chemometric models (*8−11*). Raman spectroscopy along with pattern-recognition techniques, such as discriminant analysis (DA) and soft independent modeling of class analogy (SIMCA), provide valuable qualitative information for the determination of product authenticity and quality indices for food (*12−15*).

Treatment of foods with ionizing radiation (*16*) increases the shelf life appreciably [e.g., by an extra 9 days for strawberries (*17*) and an extra 5 days for milk (*18*)]. However, irradiation can bring about chemical modifications that could affect the functional and nutritional properties of foods (*19, 20*). Electron

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spin resonance (ESR) spectroscopy and thermoluminescence (TL) has widely been employed for the detection of irradiated food (*19, 21−23*). ESR detects unpaired electrons or ions trapped in a solid matrix, such as the bone part of meat products. Similarly, TL identifies irradiated food by detecting the energy stored in the irradiated food as ions or electrons. However, these two techniques provide information only about the chemical environment of the entrapped free radicals or ions rather than the changes in the food as a whole. Detection methods that can assess the quality of processing and the chemical status of the food are ideal monitoring tools to assess process efficiency (*24, 25*). Kizil et al. (*26*) proposed the use of Fourier transform infrared (FTIR) and FT-Raman spectroscopy for the first time to characterize and classify irradiated granular starches from their nonirradiated (control) forms. In their study, discrimination of irradiated starches was attained using a hybrid partial least-squares (PLS) and canonical variate analysis (CVA) model. Discrimination was based on the multivariate statistical analysis of several special spectral regions associated with the glycosidic linkage between glucose units of starch, the water bound to the amorphous part of starch granules, and the C−H stretch and O−H stretch modes between the irradiated and control starches.

The overall goal of this study was to evaluate the potential of FT-Raman spectroscopy for rapid characterization of ionizing-energy-treated starch gels, identifying the chemical changes due to irradiation. In this study, discrimination of γ-irradiated gels prepared from corn (maize), potato, wheat, high-protein-containing maize, and high-oil-containing maize starches using FT-Raman spectroscopy along with chemometrics was explored. A hybrid PLS−CVA model was developed first to test whether FT-Raman spectroscopy can be used for the classification of irradiated starch gels based on the extent of irradiation level. Another discrimination model based on SIMCA was also developed for rapid differentiation of irradiated starch gels.

2. EXPERIMENTAL PROCEDURES

2.1. Samples and Preparation of Gels. Maize, potato, and wheat starches were purchased from Sigma Chemical Co. (St Louis, MO). The amylose contents for wheat, maize, and potato starches are 28%, 25%, and 20%, respectively. Modified starches, high-protein-containing maize starch and high-oil-containing maize starch, were obtained from Dr. Koushik Seetharaman's laboratory at The Pennsylvania State University (via Dr. Waniska at Texas A&M University). The amylose content (25%) of the modified starches is the same as that of the native maize starch because of its genetic similarity to normal corn.

Starch/water suspensions were prepared in 8-mL glass vials by dispersing aliquots of 1 g of each starch in 5 mL of distilled water. Glass vials containing starch−water suspensions were sealed and incubated in a water bath at 85 °C for 30 min and shaken vigorously. After incubation, the vials were allowed to cool to room temperature. Each starch/water suspension was gelatinized individually using 45-min time intervals to avoid or at least minimize the syneresis effect on gels during FT-Raman measurements. Gels were prepared in three separate vials.

2.2. Irradiation of Gels. A total of 15 vials of starch gels with three replicates were irradiated at 3, 5, or 10 kGy using a Gammacell 220 (MDS Nordion, Ottawa, Canada) Co-60 γ-irradiator with a dose rate of 2.1 kGy/h at the Breazeale nuclear facility at The Pennsylvania State University. The dose rate at the center of the chamber was measured using a ferrous sulfate dosimeter per MDS Nordion quality-control specifications. Irradiated starch gels were collected from the Breazeale after 6 h and placed in 10-mL Pyrex tubes before Raman measurements. It should be noted that irradiation of potato starch gels at 5 or 10 kGy disrupted the physical state of the gels. The irradiation-destroyed gels underwent a phase separation by forming a water phase over the denser gel phase.

2.3. FT-Raman Measurements. FT-Raman spectra were acquired using a Nicolet 870 spectrometer with the Raman module 32B (Madison, WI) and Nd:YAG laser operating at 1064 nm at a maximum power of 3 W. The system was equipped with an InGaAs (indium−gallium arsenide) detector, an XT-KBr beam-splitter with 180° reflective optics, and a fully motorized sample position adjustment feature. A laser output power of 1.75 W was used as required to provide a high signal-to-noise ratio. Data were collected at 16 cm^{-1} resolution with 256 scans to minimize thermal damages in gels due to the laser. Spectra were obtained in the Raman shift range between 400 and 4000 cm^{-1}. The system was operated with the OMNIC 5.1 (ThermoNicolet Analytical Instruments, Madison, WI) software, and each measurement was replicated twice. Because starch gels are not thermodynamically stable, they undergo time-dependent changes such as syneresis. To avoid or at least minimize the syneresis effect in our analyses, gel measurements were made in the order of preparation and spectra collected within 45 min. The total number of spectra collected for the samples studied was 57 (20 control, 13 3-kGy-irradiated gels, and 12 gels irradiated at 5 and 10 kGy).

2.4. Chemometrics. Two different discrimination analyses, CVA and SIMCA, were performed using the Win-DAS (Wiley, Chichester, U.K.) software package to differentiate irradiated starch gels based on the extent of irradiation. Before the analysis, area normalization and baseline correction of the spectra were done to eliminate unwanted instrumental effects on the data set. Multivariate methods such as principal component analysis (PCA) or partial least-squares (PLS) were used to compress the data to a manageable size prior to discrimination analyses.

2.4.1. Discriminant Analysis. Discriminant analysis (DA), as a subset of chemometrics, formulates a classification rule that can be used in the determination of identity or quality of an unknown sample. DA was performed with a total of 51 observations (spectra) including both control and irradiated samples. Observations were divided into four distinct groups. The first group was constructed of 14 spectra of control (three each spectra of maize, potato, wheat, and high-protein-containing maize starch gels and two spectra gels of high-oil-containing maize starch gels). The second group included 13 spectra of gels (three each of maize, potato, and wheat starch gels and two each of high-oil- and high-protein-containing maize starches) irradiated at 3 kGy, and 12 spectra of gels (three each of all starch gels except potato starch gels) irradiated at 5 and 10 kGy. PCA or PLS was first used to compress the data to obtain the scores or factors (reduced form of raw spectral data). These factors or scores were then used in CVA to classify starches within the 95% tolerance level based on the extent of irradiation.

2.4.2. SIMCA. Soft modeling can be used to develop class regression models for different values of the latent properties such as principal components to be obtained from PCA. The SIMCA method focuses on modeling the classes rather than on finding an optimal classifier. SIMCA analysis was carried out using a total of 57 spectra obtained from 20 control (four spectra from each gel type), 13 gels irradiated at 3 kGy, and 12 gels each irradiated at 5 and 10 kGy (same as provided earlier). The SIMCA model was developed using 12 nonirradiated starch gels, and the rest of the samples were used in the testing phase of SIMCA. *F*-statistics using the 95% confidence level were used for group membership decisions. In addition to *F*-statistics, SIMCA also utilizes *t*-statistics to test the normality of principal components obtained from PCA.

3. RESULTS AND DISCUSSION

The process of gelatinization causes substantial changes in both the chemical and physical nature of granular starch due to the rearrangement of intra- and intermolecular hydrogen bonding between water and starch molecules. The comparison illustrated in Figure 1 shows a discernible change in the FT-Raman spectra of granular potato and wheat starches upon gelatinization. Here, dramatic changes in the spectra of the granular starches were observed in the major carbohydrate bands between 800 and 1500 cm^{-1}. In addition, a strong Raman band of crystalline starch at 476 cm^{-1} attributed to the degree of polymerization in polysac-

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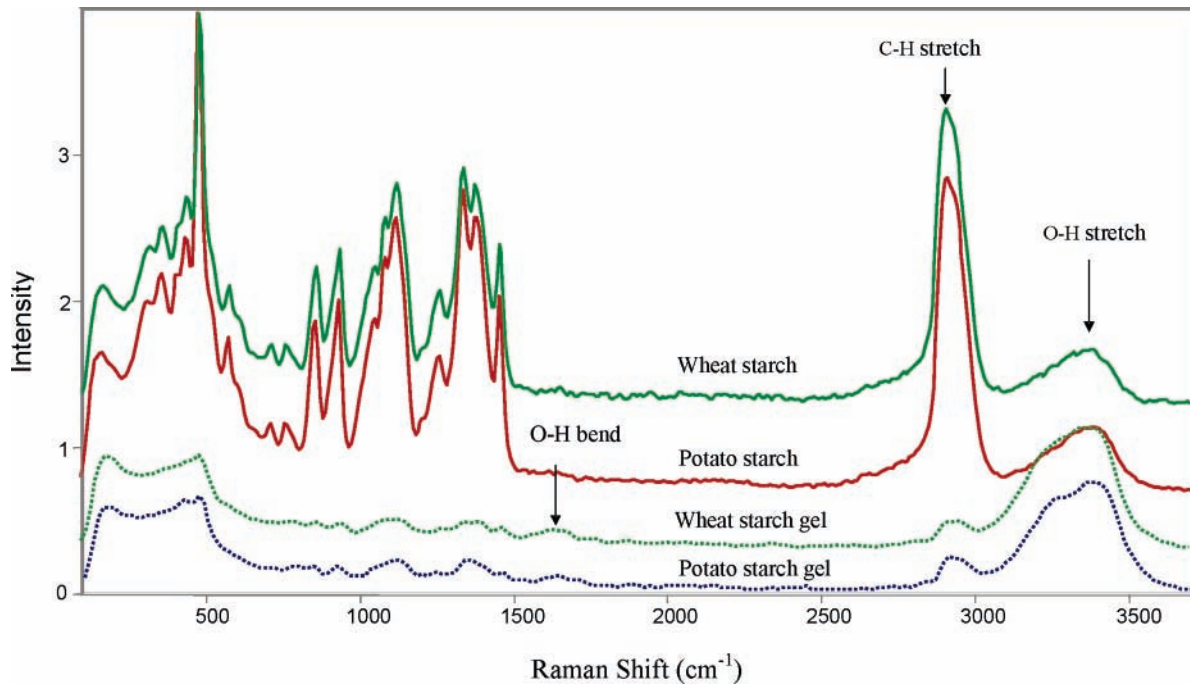*J. Agric. Food Chem.,* Vol. 54, No. 1, 2006 **15**

Figure 1. FT-Raman spectra of granular patoto and wheat starches and their gel forms.
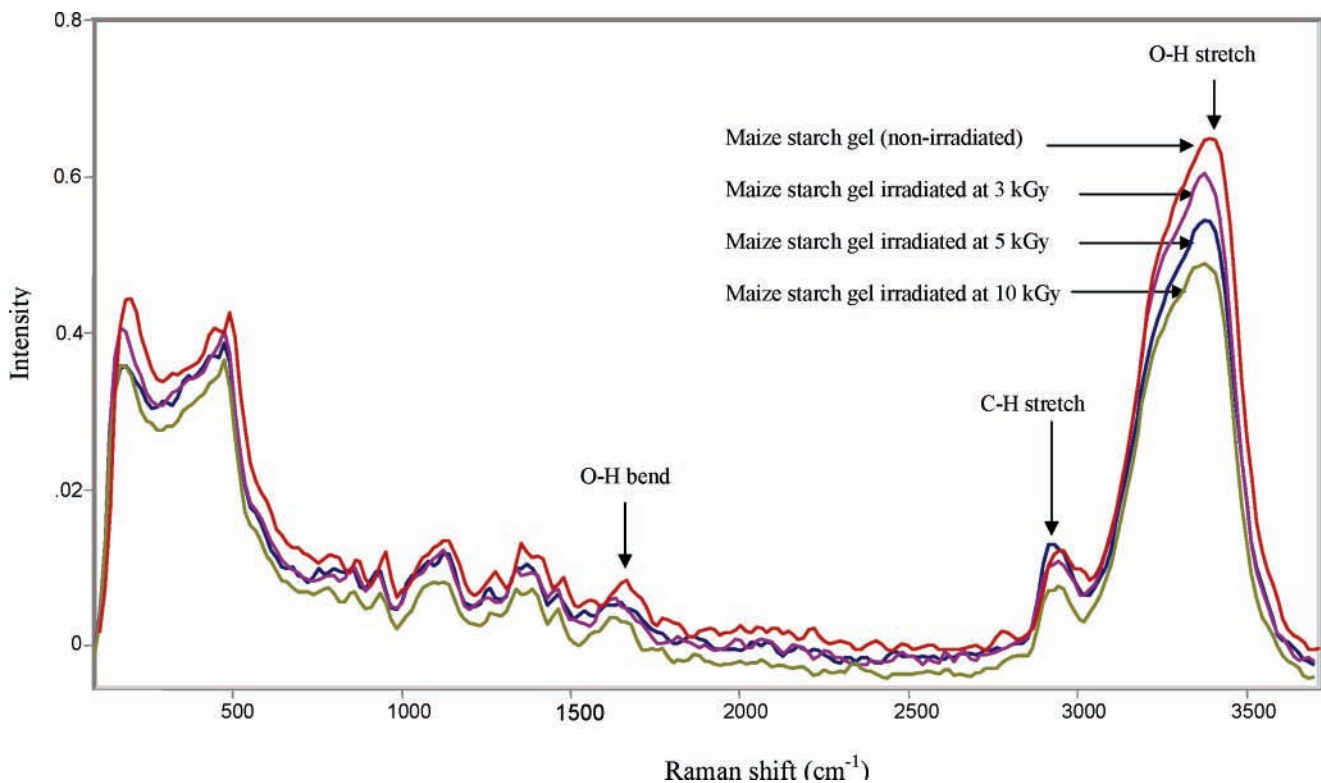

Figure 2. FT-Raman spectra of maize starch gels irradiated at three different radiation doses.

charides (*4*) underwent a significant decrease in intensity. The C–H stretch region between 2800 and 3000 cm^{-1} showed a dramatic intensity decrease upon irradiation. However, a slight broadening was detected in the O–H stretch (3000–3600 cm^{-1}) region due to the uptake of water by the starch.

In this study, the effects of γ-irrradiation on starch gels were evaluated at the molecular level by means of FT-Raman spectroscopy. Figure 2 shows the FT-Raman spectra of maize starch gel and its 3-, 5-, and 10-kGy-irradiated forms for visual evaluation of the γ-irradiation effect. The spectral changes caused by γ-radiation exposure were apparent in the O–H

stretch (3000–3600 cm^{-1}), C–H stretch (2800–3000 cm^{-1}), and bending mode of water (1600–1800 cm^{-1}).

The breaking of chemical bonds by irradiation is called radiolysis. Radiolysis yields unstable reactive agents, which are subsequently converted to stable end products. Because water is the most abundant molecule in starch gels, reactions of water radiolysis products, namely, hydroxyl radicals, free hydrogen atoms, and aqueous electrons (i.e., solvated electrons), with constituents of food are predominant in the determination of radiation-induced chemical changes in foodstuffs. The free radicals formed upon radiolysis of water trigger radiation-

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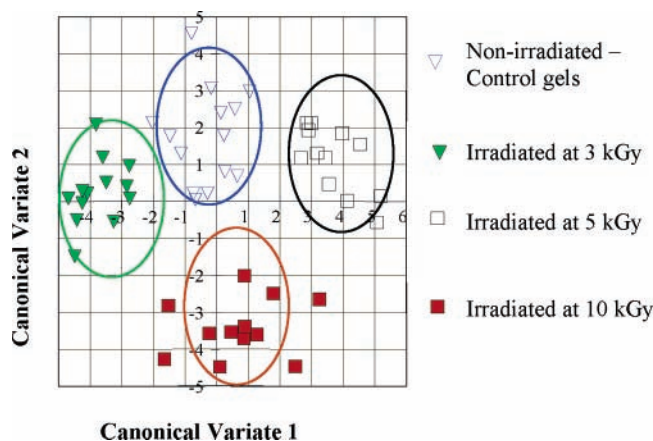
Figure 3. PLS–CVA plot for discrimination of starch gels irradiated at 3, 5, and 10 kGy using the C–H stretch and O–H bend and stretch modes.

Figure 4. PLS–CVA plot for discrimination of irradiated from nonirradiated (control) starch gels using the C–H stretch and O–H bend and stretch modes.

induced modifications in food structure through very complicated free-radical reactions (*20*). Water is not only being consumed by the radiolysis but is also formed as a reaction product through the interaction of free radicals with various molecules present in food. Hence, it would be practical to monitor water-related Raman bands for the evaluation and discrimination of irradiated gels.

The changes visually detected in the C−H stretch region of FT-Raman spectra (Figure 2) of irradiated starch gels are in good agreement with the literature. According to von Sontag (*27*), the most prominent ionizing radiation damage to carbohydrates is the liberation of a hydrogen atom from any C−H bond through the attack of hydroxyl radicals. For this reason, the C−H stretch region can also be screened to develop a discrimination model for irradiated starch gels. In this analysis, potato starch gels irradiated at 5 and 10 kGy were excluded in discrimination analyses, because a water phase separated from the gel phase at the top, losing the molecular integrity of the original gel.

Canonical variate analysis along with a proper data compression technique such as PCA or PLS was used to explore the possibility of the discrimination of irradiated gels based on the extent of irradiation regardless of the botanical origin of the starches. CVA is a supervised pattern-recognition method that separates observations (Raman spectra) into predefined groups using similarity analysis. As shown in Figure 3, a clear classification of starch gels based on the extent of irradiation was attained using a hybrid PLS−CVA approach with seven PLS factors (accounting for 92% of the cumulative variation in 51 individual spectra). The construction of PLS in the CVA analysis did provide a better separation than the implementation of PCA into CVA. A probable reason could be that PLS considers group membership information and also seeks for the most significant variations in spectral measurement, while decomposing spectral data into factors and loadings (*28*).

Another PLS−CVA model was developed to test the classification ability of the method when the samples were grouped into two observation sets (instead of four groups in the previous model), namely, control (nonirradiated) and irradiated (irrespective of the extent of applied irradiation dose). In this new PLS−CVA model, five factors from PLS were allocated in the discrimination of irradiated gels. As depicted in Figure 4, defining the observations into two groups, irradiated and nonirradiated (control) samples, resulted in a very clear separation of observations with respect to the *y* axis, the first canonical variate. Figure 4 represents a plot of the scores of the first canonical variate versus observation numbers, where irradiated
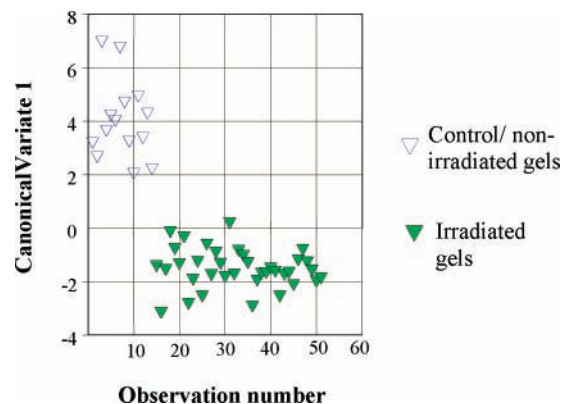
starch gels clustered at negative canonical variate score values whereas control samples were categorized by positive scores. Because the objective of CVA is to minimize within-group variance yet maximize between-group variance, discrimination can be better obtained and illustrated using a plot of the first versus second canonical variates when three or more groups are to be classified. The successful classification of irradiated starch gels from controls, as shown in **Figures 3** and **4**, is an evidence of the disruptions in the C−H and O−H bonds due to irradiation, because the discrimination models were based on the variations in Raman fingerprints corresponding to these molecular bonds.

The success in the differentiation of irradiated gels from control samples using our hybrid PLS−CVA model suggested the formulation of a class model for rapid differentiation of irradiated starch gels. The class model developed in this study was the soft independent modeling of class analogy (SIMCA) approach. SIMCA uses PCA for data reduction and involves two independent tests; *F*- and *t*-tests were used as diagnostics tools to assess the quality of the discriminatory and the modeling power of the analysis. A sufficient number of principal components from PCA are retained in SIMCA analysis accounting for most of the variations within each class (*29*). The discriminatory power of SIMCA is tested by *F*-statistics by comparing the residual variance of a sample with the mean residual variance of a class (*30*). Normal distribution (normal quantile plot) of principal components is tested using a proper statistical test, such as the *t*-test (*28*).

SIMCA can be applied to as few as 10 samples per class and presents no restriction on the maximum number of measured variables (wavenumbers) in the analysis (*29*). The latter is an important consideration in chemical sciences, because the number of measurement variables generally exceeds the number of samples in a chemical analysis. In this study, a SIMCA model (with three principal components) was constructed using 12 control (nonirradiated) starch gel spectra. Figure 5 shows the *F*-ratio plot for the SIMCA created from 12 observations. In Figure 5, the rejection lines represented by two different confidence levels, 99% (upper limit) and 95% (lower limit), were used for group decisions. Because the SIMCA was developed to create a class model to recognize only nonirradiated samples, a successful model was supposed to reject irradiated samples at a given confidence level.

The SIMCA model was tested against 8 control and 37 irradiated starch gels. All of the control starch gels were correctly predicted by the SIMCA model at the 95% confidence level. Thirty-one irradiated starch gels of 37 were rejected by the

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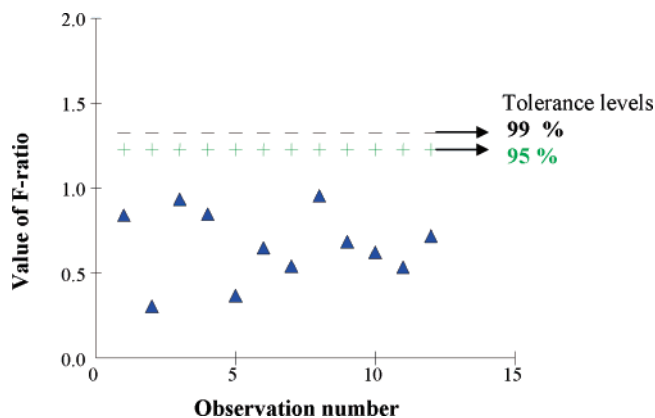
Figure 5. Testing phase of the SIMCA model used for the discrimination of irradiated starch gels with 99% and 95% tolerance levels of the *F*-test.

Table 1. Results of the SIMCA Model Created for Nonirradiated/Control Starch Gels Using 12 Observations

observation type	accepted as nonirradiated starch gel[a]	rejected[a]	percent correct classification
nonirradiated starch gels	8	0	100
gels irradiated at 3 kGy	3	10	77
gels irradiated at 5 kGy	1	11	92
gels irradiated at 10 kGy	2	10	83
total irradiated gels	6	31	84

[a] Using *F*-test at the 95% confidence level.

SIMCA *F*-test at the same confidence level. The overall success of the SIMCA in rejecting irradiated samples (i.e., assigning them as irradiated) was determined to be 84% as summarized in Table 1 for each irradiation level.

The most critical step determining the overall success of the proposed method is the proper detection of radiochemical changes in the molecular structure of starch gels by means of Raman spectroscopy. For the sugar-based food systems, hydrogen abstraction from C–H bonds was probed in the C–H stretch region of the Raman spectra. High amounts of water present in gel systems accelerate the extension of irradiation damages through interactions of hydroxyl radicals with C–H bonds of starch molecules. The Raman fingerprint of irradiated starch gels in the C–H stretch region depicts clearer variations than those of the low-moisture-containing granular starches at similar irradiation doses (*26*).

In this study, data from the most radiation-sensitive region, the C–H and O–H (due to water radiolysis) stretch regions were allocated in the discriminant models, and the control group from all five different varieties of starches were used to minimize the effect of compositional differences and gel-making properties of starches. The optimal number of PLS factors was selected in constructing the CVA models, but overfitting was avoided by keeping the number of factors lower than one-third of the differences between the number of observations used in all groups (*57*) and the number of groups (4 and 2) defined in the analysis (*31*). The class model using SIMCA was created for rapid identification of irradiated starch gels using Raman fingerprints of the C–H and O–H vibrations. The integral part in SIMCA is the training part. The success of SIMCA is dictated by a proper training phase in which samples from all different varieties should be implemented. The success rate of SIMCA used in this study could be improved by creating separate models for each starch type. However, in this study, the limited number of samples that could provide such a sample base was a factor.

4. CONCLUSION

FT-Raman spectroscopy was found to be sensitive in detecting the γ-irradiation damages on the C–H and O–H bonds of starch gels. The potential of FT-Raman spectroscopy to classify irradiated starch gels from five different courses was evaluated by means of CVA and SIMCA. The effect of chemical differences in starches from different sources was not taken into account in the discrimination analyses. The adopted FT-Raman-based approach is one of the first to demonstrate the discrimination of irradiated starch gels, as a model food, regardless of the botanical origin or modifications in starches.

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