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Determination of Quercetins in Onion (*Allium cepa*) Using Infrared Spectroscopy

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ABSTRACT: The rapid quantification of flavonoid compounds in onions by attenuated total reflectance (ATR) Fourier transform infrared (FT-IR) spectroscopy combined with multivariate analysis was evaluated as a possible alternative to high-performance liquid chromatography (HPLC) analysis. Quercetin content in onion varieties (yellow, red, and sweet) was quantified using ATR FT-IR (4000 to 400 cm^{-1}) spectroscopy and HPLC methods. Quercetin-3,4′-*O*-diglucoside (3,4′-Qdg) and quercetin-4′-*O*-glucoside (4′-Qmg) comprised >80% of the total flavonol content detected in the studied varieties. The quercetin compounds (3,4′-Qdg and 4′-Qmg) and total flavonol conjugates were quantified by HPLC, and results correlated closely with ATR-IR values (R > 0.95). Cross-validated (leave-one-out) partial least-squares regression (PLSR) models successfully predicted concentrations of these quercetins. The standard errors of cross-validation (SECV) of 3,4′-Qdg and 4′-Qmg, total quercetin, and total flavonol contents of onions were 20.43, 21.18, and 21.02 mg/kg fresh weight, respectively. In addition, supervised and unsupervised segregation analyses (principal component analysis, discriminant function analysis, and soft independent modeling of class analogue) were performed to classify onion varieties on the basis of unique infrared spectral features. There was a high degree of segregation (interclass distances > 3.0) for the different types of onion. This study indicated that the IR technique could predict 3,4′-Qdg, 4′-Qmg, total quercetin, and total flavonol contents and has advantages over the traditional HPLC method in providing a valid, efficient, and cost-effective method requiring less sample preparation for the quantification of quercetins in onion.

KEYWORDS: infrared spectroscopy, flavonoid, quercetin, onion, chemometrics

INTRODUCTION

Epidemiological studies indicate that consumption of diets high in flavonoid-containing vegetables and fruits may decrease the risk of chronic diseases[8] or delay their onset. Flavonoids are effective chelating agents of iron and copper, mediating free radical reactions[1−3] and helping to mitigate disease risk. Quercetin is the major dietary flavonol in vegetables, specifically in onions, which ranks as the second most widely consumed vegetable in the world, after tomato.[4−6] In onions, quercetin is present mainly in its conjugated forms as a glucoside. Quercetin-3,4′-*O*-diglucoside (3,4′-Qdg) and quercetin-4′-*O*-glucoside (4′-Qmg) are the two major types of quercetin conjugates in onions, whereas quercetin aglycone is also present in the outer layers and skin and there is quercetin aglycone is some parts of the bulb.[7−9]

The content of quercetin is related to various factors, such as cultivation conditions,[10−12] genetic factors controlling localization with the plant,[13−15] and storage and processing conditions.[16,17] There is interest on the part of both horticulturalists and food scientists to select onion cultivars with a high level of quercetin that is also stable and bioavailable as a means of improving the nutritional properties of onions for consumer health benefits. Current methods to quantify flavonoid compounds are through well-established high-performance liquid chromatography (HPLC) methods.[18] However, HPLC methods are time-consuming and require extraction prior to analysis, well-trained analysts, expensive instrumentation, and costly chemical reagents. In addition, an HPLC analysis from extraction to analysis takes >1 h per sample, making this method unsuitable for scanning large numbers of cultivars or onion samples for flavonol content. Also, HPLC is not adaptable for field use. Thus, other methods have been proposed to overcome those disadvantages.[19] Among these methods is near-infrared (NIR, 12000−4000 cm^{-1}) spectroscopy.[18] However, NIR lacks sensitivity and specificity.[20] In addition, flavonol analysis is negatively affected by the presence of water and fiber components that interfere with quercetin spectral features.

New analytical techniques are required in the food industry for nutraceutical quantification and prediction in intact or minimally processed food items. Fourier transform infrared (FT-IR) spectroscopy is often an appropriate technique providing a rapid and precise analysis of trace components in a complex matrix such as food. Recently, FT-IR coupled with advanced chemometric analyses was successfully used to quantify and predict lycopene and β-carotenoid concentrations in tomatoes with a coefficient of variation that was comparable to that of the classical HPLC method.[21,22] Spectroscopy-based segregation multivariate analyses can be employed to group samples on the basis of different levels of nutraceutical compounds, whereas linear regression multivariate analyses can be used to quantify nutraceutical concentrations of specific samples.[21,22] The objective of this research was to develop a methodology for the simple, rapid, and accurate determination of flavonoid compounds in onions using FT-IR spectroscopy coupled with chemometrics.

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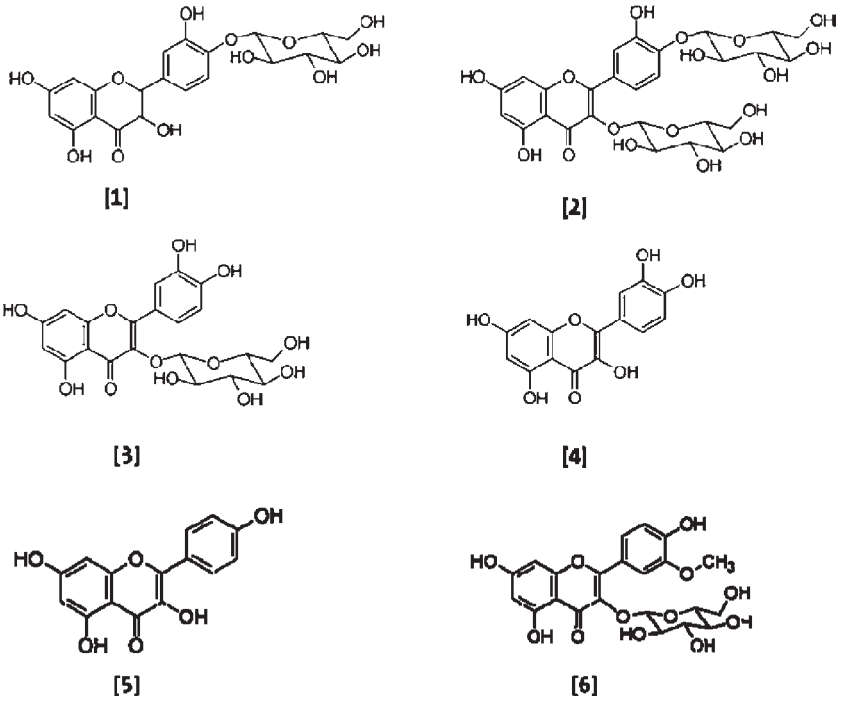

Figure 1. Chemical structures of 4′-Qmg [**1**], 3,4′-Qdg [**2**], 3-Qmg [**3**], Q [**4**], K [**5**], and 3-Img [**6**].

MATERIALS AND METHODS

Chemical Reagents. All chemicals and solvents were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). For quercetin standards, quercetin-3-*O*-glucoside (3-Qmg) (Figure 1, compound **3**), quercetin dihydrate, isorhamnetin (I), and kaempferol (K) (compound **5**) were purchased from Sigma-Aldrich. Quercetin aglycone (Q) (compound **4**) was purchased from Extrasynthese (Genay Cedex, France). Quercetin-3,4′-*O*-diglucoside (3,4′-Qdg) (compound **2**) was purchased from Polyphenols (Sandnes, Norway), and quercetin-4′-*O*-glucoside (4′-Qmg) (compound **1**) was purchased from Plantech (Berkshire, U.K.). All reagents and solvents used were of analytical or HPLC grade.

Plant Material and Sample Preparation. Red, yellow, and sweet onions were purchased from a local grocery store from the 2009 crop year and were no older than 3 months postharvest (n = 30, 10 for each type of onion). Material was selected that was free from visible blemishes or defects. Onions were stored in the dark at 4 °C until analysis, which was within 1 week of purchase. The inedible outer layers and the neck and basal parts of the onions were removed manually. The remaining tissues were pureed in a PowerBlend Duet blender/food processor (Cuisinart, East Windsor, NJ). The onion puree was then freeze-dried. The freeze-dried onion was ground in a mortar to produce a fine powder, which was then stored at 4 °C in amber vials in a dry atmosphere. Another batch of onions (n = 12, 4 for each type) was purchased separately at a different grocery store serviced by a different distributor as a way to ensure that the onions from this purchase were from separate farms and production lots. This second set of onions was used to test the prediction ability of the partial least-squares regression (PLSR) to predict flavonoid compounds. All onion samples originated from the Washington state and were weighed before and after freeze-drying.

Extraction. Extracts were prepared from 2 g of ground onion powder in 80 mL of 80% ethyl alcohol stirred at room temperature (ca. 22 °C) for 2 h void of light exposure.[7] The onion extracts were then filtered twice, first through polycarbonate 10.0 μm pore size membrane (K99CP04700, GE Water and Process Technologies, Trevose, PA) and then through a 0.22 μm MillexGP filter unit (Millipore, Carrighwahill, Ireland). Filtrate was collected in 2 mL Eppendorf tubes and placed into storage at −80 °C until analysis.[23]

Reverse Phase C18 HPLC Analysis. HPLC analyses were conducted using a Waters Alliance 2695 HPLC with a photodiode array (PDA) detector set at a wavelength of 362 nm.[15] Quercetin conjugates were separated by injecting a 50 μL sample onto a Waters μBondapak C18 (3.9 × 300 mm diameter) column coupled to a Waters μBondapak C18 guard column[23] at 20 °C. At least three separate injections were made for each sample extract. HPLC grade solvents were used in a gradient separation with solution A (98% H_2O/2% tetrahydrofuran (THF)/0.1% trifluoroacetic acid (TFA)) and solution B (100% acetonitrile) at a flow rate of 1 mL/min[24] over a 35 min total run time. The optimal separation was obtained with modified gradient as follows to optimize peak separation: isocratic 17% B for 2 min; gradient to 90% B for 20 min; gradient to 95% for 1 min: isocratic 95% B for 2 min; gradient to 17% B for 2 min; and isocratic 17% B for 8 min to reequilibrate the column.[23]

Quercetin Quantification. Standard curves of each quercetin compound were determined by HPLC with standards run every 10 samples. The standard curves were established to resolve the ranges of quercetin concentration in onions as reported by others.[1,2,21] Serial dilutions ranged in a concentration from 0.00 to 200.00 μg/mL for each standard. Compounds analyzed here were (1) 3,4′-Qdg (compound **2**) + 4′-Qmg (compound **1**) (main quercetin glucosides, accounting for ∼80% of total flavonols in onions); (2) total quercetin (3,4′-Qdg + 4′-Qmg + 3-Qmg + Q) (compound **2** + compound **1** + compound **3** + compound **4**); (3) total flavonols (3,4′-Qdg + 4′-Qmg + 3-Qmg + Q + K + isorhamnetin-3-*O*-glucoside (3-Img) (compound **2** + compound **1** + compound **3** + compound **4** + compound **5** + compound **6**); HPLC retention times for these compounds ranged between ∼5 and ∼25 min).[23]

FT-IR Instrumentation and Spectral Measurement. FT-IR spectra of onion extracts were recorded at room temperature (ca. 22 °C) using a Nicolet Avatar 380 spectrometer (Thermo Electron Inc., San Jose, CA) scanning over the frequency range of 4000−400 cm^{-1} at a resolution of 4 cm^{-1}. Spectra were collected by using rapid scan software

running under OMNIC (Nicolet, Madison, WI). The spectrum of each sample was an average of 128 scans with two spectra taken per aliquot. The internal reflection element was a zinc selenide (ZnSe) horizontal attenuated total reflectance (HATR) through plate crystal with an aperture angle of 45°.

Ethanolic extracts were allowed to equilibrate to room temperature (ca. 22 °C) before scanning. The ethanolic aliquots of 20 μL each were uniformly spread directly onto the HATR crystal cell before spectral measurement. Four aliquots were prepared from each onion extract and tested in duplicate, for a total of eight spectra for each onion sample (n = 30). The aliquots were dried to form a uniform layer on the surface of crystal cell (ca. 22 °C), which occurred within approximately 5 min. Drying the sample into a film removed the interference of both ethanol and water from the spectra and increased the intensity and resolution of the remaining spectral bands.[25] The same instrumental background settings were maintained for each set of samples, and the crystal cell was cleaned between spectral collections using 0.1% (w/v) Alconox solution (Alconox Inc., New York, NY).

Precision and Accuracy of Analytical Methods. The precision of HPLC and FT-IR methods was determined by repeatability (intraday) and intermediate precision (interday). Repeatability was evaluated by assaying samples with the same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days. Accuracy was determined in the same conditions as precision. Precision was expressed as relative standard deviation (RSD) and accuracy as the agreement between the measured and the true values by standard addition. To check the precision and accuracy of HPLC and FT-IR methods, intra- and interday, respectively, quercetin concentration levels of 10, 50, and 100 μg/mL were used. Significant differences ($p < 0.05$) between HPLC and FT-IR methods for precision and accuracy were determined by one-way analysis of variance (ANOVA) followed by t test using Matlab2010a (Math Works Inc., Natick, MA).

Data Preprocessing. Infrared spectra were first preprocessed by EZ OMNIC 7.1a (Thermo Electron Inc., Lafayette, CO). Then, automatic baseline correction was performed to flatten baseline, followed by a smoothing of 5 (Gaussian function of 9.643 cm^{-1}). The preprocessed spectra were transferred into Excel (Microsoft Inc., Redmond, WA). Second -derivative transformations using a 9-point Savitzky−Golay filter and wavelet transforms (with a scale of 7) were employed for spectral processing in Matlab (Math Works Inc.) to enhance the resolution of superimposed bands and to minimize problems from unavoidable baseline shifts.

Spectral Reproducibility Analyses. The reproducibility of FT-IR spectra from independent experiments was investigated by calculating D_{y1y2} according to following equations. In the equations, y_{1i} and y_{2i} are signal intensities of two different spectra, whereas $\overline{y_1}$ and $\overline{y_2}$ are average values of signal intensities of two different spectra; n represents the data points in the selected wavenumber region. D_{y1y2} ranges from 0 to 2000. The lower the value, the better the reproducibility of spectra, being 0 when spectral ranges are identical, 1000 for completely noncorrelated spectra, and 2000 for completely negatively noncorrelated spectra.[26]

$$r_{y1y2} = \frac{\sum_{i=1}^{n} y_{1i}y_{2i} - n\overline{y_1}\,\overline{y_2}}{\sqrt{\sum_{i=1}^{n} y_{1i}^2 - n\overline{y_1}^2}\sqrt{\sum_{i=1}^{n} y_{2i}^2 - n\overline{y_2}^2}}$$

$$D_{y1y2} = (1 - ry_1y_2) \times 1000$$

Multivariate Analyses. Chemometric models based on processed spectra, including cluster analysis (principal component analysis, PCA), dendrogram analysis (discriminant function analysis, DFA), class analogue analysis (soft independent class of analogues, SIMCA), partial least-squares regression (PLSR), and loading plot analysis were established.

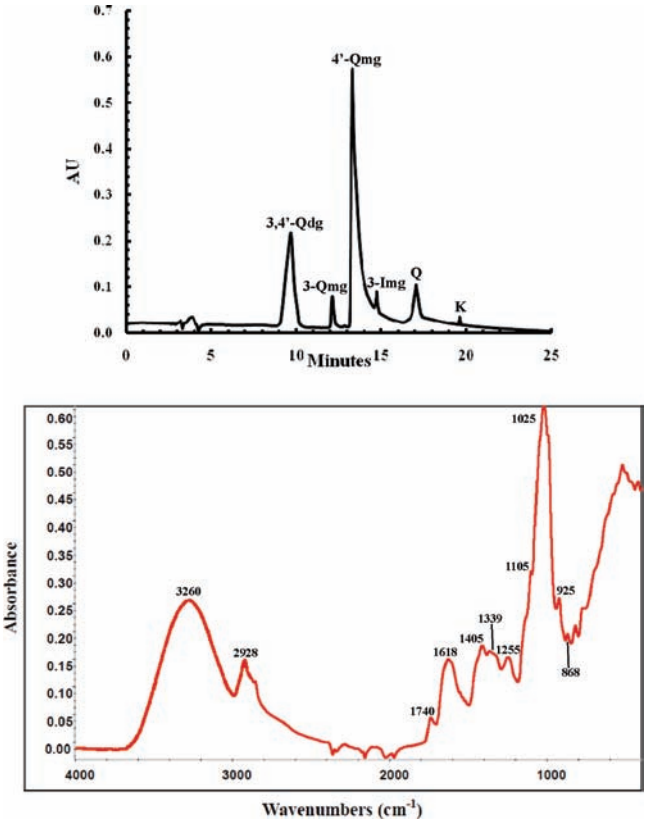

Figure 2. Representative HPLC chromatogram (left) and FT-IR spectrum (right) of yellow onion (*Allium cepa*) extract.

Segregation Model Analyses. PCA is a vector space transformation technique for reducing a data set into its predominant features, segregating samples within a data set into discrete clusters. PCA determines which major factors affect the differences observed in the spectral features among samples and then uses this information to construct a two- or three-dimensional model to segregate samples on the basis of selected variances.[22] DFA can construct branched dendrogram structures using prior knowledge of the composition of a biological sample. Both PCA and DFA employ the same PCs for model analyses.[27] SIMCA is a supervised classification method. It is extensively employed to classify samples on the basis of how similar they are to samples in the training set.[25] Although analogy is important, there is only a "pass" or "fail" option in a SIMCA model based on whether a test sample has a predetermined similarity to the samples within the training set. The degree of similarity is based on the biochemical composition of a food matrix, and it is usually less intensive than a PCA or DFA classification for differentiating different samples.[25] The combination of different chemometric models could improve and finally validate the properties of test samples.

Linear Regression Model Analyses. The PLSR was employed for quantitative analysis using Matlab. A total of eight spectra from each sample (n = 30) were used to establish the calibration model. A leave-one-out cross-validation was performed to evaluate the prediction power of the model by removing one standard from the data set at a time and applying a calibration to the remaining standards.[21] To establish a PLSR model, the first step was to choose the optimal number of latent variables. Being able to establish a robust model and having a sufficient spectral library are necessary for predicting which spectral features are correlated to each reference parameter.

Predicting Flavonoid Contents in Onions. An independent set of randomly selected onion samples (n = 12) that included three onion varieties used as a prediction set for PLSR modeling was analyzed to predict flavonol contents. The flavonol contents were determined by the HPLC reference method. FT-IR spectra were measured for samples as described above and used to test the predictive ability of the PLSR calibration model for flavonol content.

RESULTS AND DISCUSSION

Spectral Analyses. *Spectral Band Assignments.* The IR spectral features of yellow onion are shown in Figure 2. The bands between the wavenumbers of 1800 and 750 cm^{-1} (FT-IR "fingerprint" region) reflect the primary biochemical and macronutrient components, such as carbohydrates, lipids, proteins, and nucleic acids. The band at 1740 cm^{-1} is assigned to >C═O stretch of esters.[25] The distinctive band at 1618 cm^{-1} is assigned to ring C−C stretch of phenyl.[28] This feature would correlate well with high levels of flavonoid compounds in onions. The band at 1405 cm^{-1} is assigned to CH_3 asymmetric deformation.[29] The band at 1339 cm^{-1} is assigned to in-plane C−O stretching vibration combined with the ring stretch of phenyl.[28] The minor band at 1255 cm^{-1} is assigned to amide III (random coil) for protein.[30] The wavenumber region between 1200 and 950 cm^{-1} contains a functional group mainly from carbohydrate.[25] The band at 1105 cm^{-1} is from carbohydrate, whereas the bands at 1025 and 985 cm^{-1} are assigned to the vibrational frequency of $-CH_2OH$ groups of carbohydrates and OCH_3 from polysaccharides−cellulose, respectively.[30] The bands at both 925 and 868 cm^{-1} are assigned to DNA structure.[29] For the higher wavenumbers, the band at 3260 cm^{-1} is assigned to N−H stretching of proteins and O−H stretching of carbohydrates and water, whereas the band at 2928 cm^{-1} is assigned to CH_2 antisymmetric stretch of methyl groups mainly from lipids.[25] The raw spectral features of all three onion varieties were similar (data not shown). The detection limit is 0.008% in the current study using ATR FT-IR spectroscopy for quercetin determination by spiking known amounts of quercetin into onion samples.

Spectral Reproducibility Analyses. The reproducibility of FT-IR spectra from independent experiments was calculated using the Pearson coefficient (expressed as D_{y1y2} value). Mean D values between 7 and 10 are considered to be normal when the first or second derivative of samples is analyzed.[26] The D value of FT-IR spectra is related to the wavenumber region (window) chosen.[29] First, five windows were selected to calculate the D value: (1) whole wavenumber region (3300−900 cm^{-1}, w_1); (2) 3000−2800 cm^{-1} (fatty acids, w_2); (3) 1800−1500 cm^{-1} (proteins and peptides, w_3); (4) 1500−1200 cm^{-1} (mixed region of proteins, fatty acids, and other phosphate-carrying compounds, w_4); and (5) 1200−900 cm^{-1} (carbohydrate, w_5). High D values were obtained from w_1 (13.75 ± 3.12 to 18.95 ± 3.61) and w_2 (13.18 ± 2.36 to 15.45 ± 3.25), whereas low D values were obtained from w_3 (8.24 ± 0.52 to 8.87 ± 0.34), w_4 (7.41 ± 0.18 to 8.07 ± 0.34), and w_5 (9.15 ± 0.29 to 9.43 ± 0.07). In addition, window combinations were evaluated to check D values of complex wavenumbers. The wavenumber region of 1800−900 cm^{-1} was used, with corresponding D values of 8.14 ± 0.46 to 8.91 ± 0.28. On the basis of these low D values, the use of combined wavenumber regions (1800−900 cm^{-1}) to employ the chemometric analyses was appropriate.

Precision and Accuracy. The precision and accuracy of HPLC and FT-IR methods were determined. For HPLC, intraday RSD ranged from 0.001 to 0.06%, and interday RSD ranged from 1.45 to 5.31%; intraday error ranged from 0.69 to 2.17%, and interday error ranged from 1.35 to 8.23%. For FT-IR, intraday RSD ranged from 0.001 to 0.045%, and interday RSD ranged from 1.37 to 4.18%; intraday error ranged from 0.75 to 1.99% and interday error ranged from 2.47 to 7.49%. There was no significant (p > 0.05) difference between HPLC and FT-IR methods for either precision or accuracy.

Quantitative Analysis of Onion Flavonoids. *Quantitative Analysis by HPLC.* In preliminary experiments, the extraction efficiency of different ethanol and methanol concentrations of 20, 40, 60, 80, and 100% was tested. Quercetin levels were up to 15% higher using an extraction of 80% ethanol or methanol compared to other concentration treatments (data not shown). This was in agreement with previous studies indicating that the extraction solution used is important in the recovery of flavonoid compounds due to its polarity.[7] Furthermore, a water−ethanol extract could speed spectral measurements and provide greater resolution of spectral features compared to the pure ethanolic extract due to more uniform film formation (data not shown).

HPLC chromatograms revealed six peaks in a profile similar to those reported in other studies quantifying quercetin in onion.[8,24] A typical chromatogram showing the elution of quercetins between 5.0 and 25.0 min appears in Figure 2. This region represents total flavonol content in onions according to previous works.[23,24] Four minor flavonol components were identified as quercetin-3-*O*-glucoside (3-Qmg, compound **3**; retention time 12.14 min), isorhamnetin-3-*O*-glucoside (3-Img, compound **6**; retention time 14.75 min), quercetin aglycone (Q, compound **4**; retention time 17.05 min), and kaempferol (K, compound **5**; retention time 19.62 min). Combined, these four compounds accounted for 7−15% of total flavonol content in all three onion varieties. Two large peaks occurring at retention times of 9.69 and 13.30 min were identified as quercetin-3,4′-*O*-diglucoside (3,4′-Qdg, compound **2**) and quercetin-4′-*O*-glucoside (4′-Qmg, compound **1**), respectively. Combined, 3,4′-Qdg (compound **2**) and 4′-Qmg (compound **1**) accounted for 85−87% of total flavonol compounds, depending on the onion variety (Table 1).

Total quercetin concentrations by HPLC analyses, a summation 3,4′-Qdg (compound **2**), 3-Qmg (compound **3**), 4′-Qmg (compound **1**), and Q (compound **4**) peaks, ranged from 367.81 to 653.19 mg/kg fresh weight (fwt) and represented >85% of total flavonol content (Table 1). Our results for total quercetin concentrations in onions were significantly lower than those reported by Price and Rhodes[17] of 1369−1778 mg/kg fwt for total quercetin. However, our values were close to those of Patil et al.,[7] with a range of 54.34−286.40 mg/kg fwt and a mean of 513.3 mg/kg fwt reported by Lombard et al.[23] In addition, other researchers have verified that 3,4′-Qdg (compound **2**) and 4′-Qmg (compound **1**) are the two major quercetin conjugates and account for >80% of total flavonol in onions.[13−19,23,24,31]

The variations in flavonol concentrations may due to various factors. Cultivation conditions play a key role in determining the flavonoid concentrations in onions.[10−12] The biosynthesis of flavonoids was mainly affected by different cultivation conditions, such as weather conditions, plant location, and harvest period.[16] Genetics also play a role.[31] Flavonoids are generally found at higher concentrations in the outer layers of fruits and vegetables; therefore, peeling results in great losses.[11,17] This also partially explained the lower quercetin contents determined in the current study because the inedible outer layers were removed, whereas some other studies have employed the whole onion during

Table 1. Quercetin Concentration in Onion (*Allium cepa*) Variety Quantified with HPLC-PDA (362 nm)

variety	3,4′-Qdg (mg/kg fwt)	4′-Qmg (mg/kg fwt)	3,4′-Qdg + 4′-Qmg (mg/kg fwt)	% TF	total quercetin (mg/kg fwt)	% TF	TF (mg/kg fwt)
yellow onion	183.92 ± 23.93	236.6 ± 37.55	420.52 ± 56.06	87.82%	426.54 ± 56.27	89.08%	478.83 ± 56.73
sweet onion	168.93 ± 27.70	257.04 ± 45.74	425.97 ± 70.76	85.41%	434.03 ± 69.29	87.03%	498.73 ± 65.92
red onion	253.67 ± 18.88	374.16 ± 18.00	627.83 ± 30.87	84.61%	634.31 ± 31.08	85.49%	742.00 ± 33.89

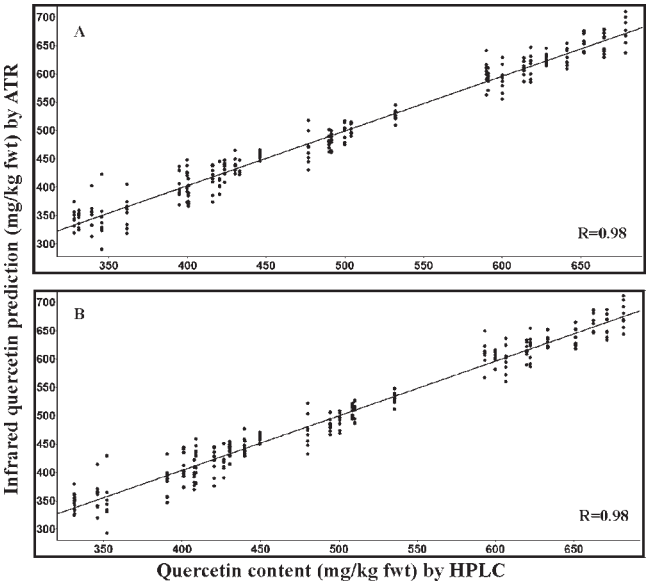

Figure 3. Correlation between cumulative quercetin conjugates of onion ethanolic extracts separated by HPLC and IR prediction values: (A) 3,4′-Qdg + 4′-Qmg; (B) total quercetin (3,4′-Qdg + 3-Qmg + 4′-Qmg +Q). n = 30 bulbs.

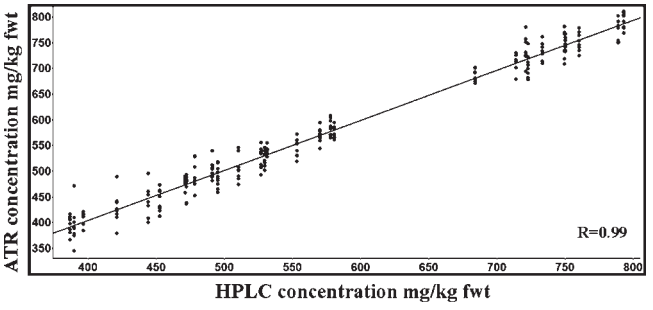

Figure 4. Correlation between cumulative flavonol compounds of onion ethanolic extracts separated by HPLC and IR prediction values: 3,4′-Qdg, 3-Qmg, 4′-Qmg, 3-Img, Q, and K. n = 30 bulbs.

flavonoid extraction.[32] The higher levels of flavonoids in the outer skin serve to protect the plant from ultraviolet radiation damage. Flavonoids present in the peel are mainly aglycones due to flavonol glucoside hydrolysis occurring during peel formation.[5] Gennaro et al. reported that red onions contained 79% of the original total content of 4′-Qmg (compound **1**) after peeling and that 3,4′-Qdg (compound **2**) and 4′-Qmg (compound **1**) were unaffected by chopping of onions.[33] In general, the onion bulb contains a broad range of quercetin concentrations, with increasing concentrations of quercetin glucosides from the inner to the outer scales.[3]

Quantitative Analysis by FT-IR. PLSR using the wavenumbers from 1800 to 900 cm^{-1} was employed to establish linear regression between HPLC reference values and FT-IR spectral features for flavonoids quantification (Figures 3 and 4). All parameters derived from the cross-validated (leave-one-out) PLSR model results are shown in Table 2. Second-derivative transformation of the spectral measurements improved the quantitative analysis by resolving overlapped bands and limiting variations in spectral baselines, which improved the chemometric model performance.[25] A good PLSR model should have a high value for regression coefficient ($R > 0.95$) and a low standard error of both calibrated and cross-validated chemometric models.[25] In addition, to control the latent variable <10 is desired because calibrated model overfitting could cause severe imprecision in the prediction.[27] The estimated contents of major quercetin (3,4′-Qdg + 4′-Qmg) (compound **2** + compound **1**), total quercetin (3,4′-Qdg + 4′-Qmg + 3-Qmg + Q) (compound **2** + compound **1** + compound **3** + compound **4**), and total flavonol compounds measured by FT-IR spectroscopy showed good correlation with the reference HPLC analysis. The standard error of calibration and standard error of cross-validation were less than 20.43, 21.18, and 21.02 mg/kg fwt of extracts for quantification of major quercetin, total quercetin, and total flavonol, respectively (Table 2).

The PLSR models were employed to predict the concentrations of 3,4′-Qdg (compound **2**), 4′-Qmg (compound **1**), total quercetin, and total flavonol in an independent set of onion extracts (n = 12). FT-IR predictions were comparable to the HPLC reference values, as shown in Table 3. Better reproducibility among measurements (coefficient of variability, CV) was obtained with the FT-IR method as compared to HPLC analysis. In general, the CVs for these three biological parameters in spectroscopy-based PLSR models were similar to each other.

Chemometric Classification of Onion Samples. Unsupervised PCA and supervised DFA were employed simultaneously to establish cluster and dendrogram chemometric models for onion sample segregation on the basis of spectral features. First, PCA was performed to plot class projection using the first three PCs. The two-dimensional cluster segregation is shown in Figure 5. Three varieties of onion samples were well segregated from each other, forming tight clusters with interclass distances ranging from 11.36 to 39.52 based on Mahalanobis distance measurements computed between the centroids of classes. Clusters with interclass distance values >3 are considered to be significantly different from each other.[22] In addition, a dendrogram chemometric model was derived on the basis of selected PCs from the PCA model and prior knowledge of samples using hierarchical cluster analysis (Figure 5). Spectral features of three different varieties of onions were distinctive, with no samples misclassified (n = 30).

SIMCA was continuously performed to validate the two segregation models. Class analogue results are shown in Table 4, and a >90% correction rate for data classification was achieved.

In conclusion, the FT-IR technique allowed the development of linear regression models and segregation models for the quantitative and qualitative analysis of quercetin in onion extracts. Our PLSR models for the quantification of the main

Table 2. PLSR Models (1800−900 cm^{-1}) for Determination of Specific Analytes in Onion (*Allium cepa*)

analyte	ref range (mg/kg fwt)	no. of samples	latent variables	*R*	SECV (mg/kg fwt)	*R* calcd	SEC (mg/kg fwt)
3,4′-Qdg + 4′-Qmg	327.98−678.68	240	6	0.98	20.43	0.99	17.47
total quercetin	331.19−681.94	240	6	0.98	21.18	0.99	17.98
total flavonol	386.59−793.49	240	5	0.99	21.02	0.99	16.41

Table 3. PLSR Model Predicted Content of 3,4′-Qdg, 4′-Qmg, Total Quercetin, and Total Flavonol in Onion (*Allium cepa*)

analyte	sample	ref value (mg/kg fwt)	SD	CV (%)	IR predicted value (mg/kg fwt)	SD	CV (%)
3,4′-Qdg	yellow onion	165.32	6.78	4.10	169.33	3.56	2.10
	sweet onion	141.28	5.29	3.74	137.95	4.57	3.31
	red onion	249.71	9.98	4.00	243.24	8.29	3.41
4′-Qmg	yellow onion	263.27	8.23	3.13	261.07	3.98	1.52
	sweet onion	249.86	7.36	2.95	253.38	4.21	1.66
	red onion	380.83	11.95	3.14	384.29	7.48	1.95
total quercetin	yellow onion	435.75	9.25	2.12	439.89	9.02	2.05
	sweet onion	402.34	8.77	2.18	397.13	6.31	1.59
	red onion	636.18	13.28	2.09	642.18	10.26	1.60
total flavonol	yellow onion	476.62	14.23	2.99	485.58	11.39	2.35
	sweet onion	481.73	15.63	3.24	473.07	9.95	2.10
	red onion	774.56	19.80	2.50	783.64	13.94	1.78

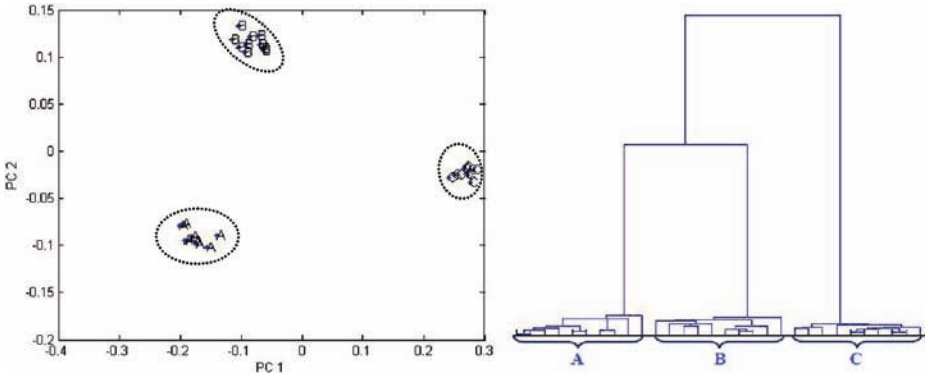

Figure 5. Segregation models: principal component analysis (left) and discriminant function analysis (right) results for yellow onion (A), sweet onion (B), and red onion (C).

Table 4. SIMCA Classification Results for Each Sample Category Compared to Other Sample Categories

variety	no. of correctly classified spectra	% of correctly classified spectra
yellow onion	73	91.25
sweet onion	76	95.00
red onion	77	96.25

quercetin, total quercetin glycosides, and total flavonols showed good performance statistics. PLSR models generated from mathematically transformed infrared spectral data gave correlation coefficients (*R* value) of >0.95 between the FT-IR predicted and HPLC reference values and SECV 20.43, 21.18, and 21.02 mg/kg of onion extracts for main quercetin, total quercetin, and total flavonol, respectively. This technique provides fast analysis of nutraceuticals (phenolics) with minimal sample preparation, little personnel training, simple data acquisition, and immediate prediction of concentration.

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