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Determination of Total Phenolic Content and Antioxidant Activity of Garlic (*Allium sativum*) and Elephant Garlic (*Allium ampeloprasum*) by Attenuated Total Reflectance−Fourier Transformed Infrared Spectroscopy

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ABSTRACT: The total phenolic contents and antioxidant activities of garlics from California, Oregon, Washington, and New York were determined by Fourier transform infrared (FT-IR) spectroscopy (400−4000 cm^{-1}). The total phenolic content was quantified [Folin−Ciocalteu assay (FC)] and three antioxidant activity assays, 2,2-diphenyl-picrylhydrazyl (DPPH) assay, Trolox equivalent antioxidant capacity (TEAC) assay, and ferric reducing antioxidant power (FRAP), were employed for reference measurements. Four independent partial least-squares regression (PLSR) models were constructed with spectra from 25 extracts and their corresponding FC, DPPH, TEAC, and FRAP with values for 20 additional extracts predicted ($R > 0.95$). The standard errors of calibration and standard error of cross-validation were <1.45 (TEAC), 0.36 (FRAP), and 0.33 μmol Trolox/g FW (DPPH) and 0.55 mg gallic acid/g FW (FC). Cluster and dendrogram analyses could segregate garlic grown at different locations. Hydroxyl and phenolic functional groups most closely correlated with garlic antioxidant activity.

KEYWORDS: Garlic, antioxidant, phenolic, FT-IR, chemometrics, PLSR, DF-PCA

INTRODUCTION

Epidemiological studies show an inverse correlation between garlic (*Allium sativum*) consumption and the reduced risk of coronary disease and cancer.[1] *Allium* species contain a wide range of chemicals possessing both antioxidant and oxidant properties. Elephant garlic (*Allium ampeloprasum*) is more closely related to the leek than to ordinary garlic. A single clove of elephant garlic can be as large as a whole bulb of ordinary garlic. It is much less intense and sweeter and is often described as "garlic for people who don't like garlic".

Both phenolic and organosulfur compounds are bioactive components contributing to antioxidant activity in *Allium* species.[1−3] The antioxidant profiles of thiosulfinates (allicin and ajoene)[4] and diallyl sulfides[5] have been quantified by both enzymatic assays and nonenzymatic assays. In vitro studies demonstrated that garlic exhibits a dose-dependent radical capture response.[1]

The total antioxidant activity of vegetables and fruits has been extensively studied using various chemical-based assays, including 2,2-diphenyl-picrylhydrazyl (DPPH), Trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), and others. Recently, cell culture assays and animal bioassays have become more common as a means to more accurately determine the impact of these compounds on cell and organ function and growth.[6] However, all of these assays are time-consuming, and developing an alternative method to substitute or at least validate the traditional "wet chemistry" methods is important if a large number of samples are to be evaluated. Infrared spectroscopy provides a unique advantage of simple sample preparation while retaining satisfactory precision and sensitivity. Infrared spectroscopy combined with chemometrics has been widely used in the detection of food toxicants, food adulterants, and foodborne pathogens.[7]

The application of infrared spectroscopy to quantitate and predict phenolic content and total antioxidant activity is a new area. Near infrared spectroscopy has been used to quantify flavonoid content, total phenolic content (TPC), and antioxidant activity of rice grain.[8] In another study, the antioxidant activity of green tea was studied using near-infrared spectroscopy.[9] In the case of midinfrared spectroscopy, the antioxidant activities of red wine[10] and fruit extracts[11] were quantified by Fourier transform infrared (FT-IR) spectroscopy (400−4000 cm^{-1}). To date, there is little work using infrared spectroscopy to quantify phenolic compounds and antioxidant activities in vegetables. In the current study, we quantify the TPCs and antioxidant activities of garlics from different locations and verify which functional groups contribute the most to antioxidant activity of garlic through the use of spectroscopic chemometric models. This study may provide a new approach to understanding the free radical quenching mechanisms of antioxidants in vegetables.

MATERIALS AND METHODS

Chemicals and Reagents. Folin−Ciocalteu reagent, DPPH, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) diammonium salts (ABTS), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), 2,2′-azobis(2-amidinopropane)dihydrochloride (ABAP), hydrochloric acid (HCl), ferric chloride ($FeCl_3$), acetate, and

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gallic acid were obtained from Sigma-Aldrich (St. Louis, MO). Sodium carbonate (Na_2CO_3), methanol, potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), sodium hydroxide, and HCl were purchased from J. T. Baker Inc. (Phillipsburg, NJ). All reagents and solvents used were analytical or high-performance liquid chromatography (HPLC) grade.

Preparation of Garlic Samples. Garlic grown in four states (Washington, Oregon, California, and New York) and elephant garlic (from California) were purchased from local grocery stores on two separate occasions. Bulbs were selected at random from each lot purchased with all cloves from a single bulb composited into a single sample (N = 25 for each type of garlic). For elephant garlic, individual cloves were selected for testing (N = 25). All garlics were from the 2009 crop year and no older than 4 months postharvest when used in these experiments. Bulbs and cloves were selected that were free from visible blemishes or defects. Garlic bulbs were manually peeled and chopped.

Extraction. Two grams of chopped fresh garlic was extracted with 15 mL of 70% methanol under magnetic stirring for 2 h at room temperature (ca. 22 °C).[1,2] The extract was centrifuged at 4000*g* for 20 min, and the supernatant was filtered through polycarbonate 0.4 μm pore size membrane (GE Water & Process Technologies, Trevose, PA). The extraction procedure was repeated three times, and supernatants were pooled together. All extractions were performed in triplicate, avoiding light exposure during the extraction process, and following extraction, they were stored in the dark at 4 °C within an hour. The weight of dry matter of the extracts was determined and standardized to 1 mg/mL by measuring the absorbance at 515 nm and diluting all extracts to the same optical density with 70% methanol prior to collection of spectra.

Determination of TPC. The TPC of each extract was determined in duplicate by the Folin−Ciocalteu procedures[12] with minor changes. In brief, Folin−Ciocalteu reagent was diluted 10-fold with deionized water. The 70% methanolic garlic extracts (0.1 mL) were mixed with 0.75 mL of the diluted Folin−Ciocalteu reagent and incubated for 10 min at room temperature (ca. 22 °C). Then, 0.75 mL of 2% Na_2CO_3 (w/v) solution was added. The mixture was kept in the dark (ca. 22 °C) for 45 min before measuring the absorbance at 765 nm using an Ultrospec 4000 UV/Visible spectrophotometer (Pharmacia Biotech, Cambridge, United Kingdom) against a blank, containing deionized water instead of sample extract. TPC values were determined from a calibration curve prepared with a series of gallic acid standards (0, 5, 10, 15, 20, 30, and 40 mg/L). Results are expressed as mg of gallic acid equivalents/g fresh weight (mg GAE/g FW).

DPPH Assay. The antioxidant activity of garlic extracts was measured using a DPPH method previously described[5,8,13] with the free radical DPPH, with minor revisions. Aliquots (0.1 mL) of diluted extracts were added to 1 mL of DPPH solution, and the absorbance of the DPPH solution was determined at 515 nm after 30 min of incubation at room temperature (ca. 22 °C).[7] Seventy percent methanolic solutions of Trolox in a range of 0−500 μmol/L were used for calibration to compare the antioxidant activity of garlic extracts. The antioxidant activity of the sample was expressed as μmol Trolox equivalents/gram fresh weight sample (μmol Trolox/g FW).

TEAC Assay. This assay is based on the decolorization of the radical cation 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) $ABTS^+$ after reduction to ABTS. Spectrophotometric methods were performed as previously described.[14] A phosphate buffer was prepared by mixing 818 mL of 66 mmol/L Na_2HPO_4 with 182 mL of 66 mmol/L KH_2PO_4 solution and 150 mmol of NaCl. For the daily preparation of the ABTS solution, 0.5 mL of 20 mmol/L ABTS in the phosphate buffer was mixed with 100 mL of ABAP (2.5 mmol/L) in the phosphate buffer and heated for 15 min in a 60 °C water bath. The reaction was initiated by adding 1.96 mL of the $ABTS^+$ solution to 40 μL of the garlic sample or Trolox standard solution or 40 μL of 70% methanol in water as a control. The mixture was allowed to stand for 6 min at room temperature (ca. 20 °C). The absorbance was then measured at 734 nm. Seventy percent methanolic solutions of Trolox in a range of 0−500 μmol/L were used for calibration. TEAC radical scavenging results were expressed as μmol Trolox equivalents/gram fresh weight sample (μmol Trolox/g FW).

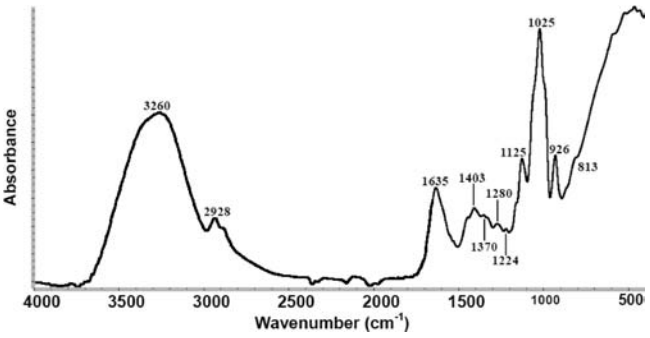

Figure 1. Representative FT-IR raw spectra of garlic.

FRAP Assay. This method is based on the increase in absorbance at 593 nm due to the formation of tripyridyl-*S*-triazine complexes with Fe^{2+} [TPTZ-Fe(II)] in the presence of a reducing agent.[15] The FRAP reagent was prepared from 2.5 mL of a TPTZ solution (10 mmol/L) in HCl (40 mmol/L) and 2.5 mL of a $FeCl_3$ solution (20 mmol/L) mixed with 25 mL of an acetate buffer (0.3 mol/L, pH 3.6). For the determination of the antioxidant activity, the FRAP reagent (1.5 mL) was mixed with 100 μL of deionized water and 100 μL of the garlic extracts, Trolox standard, or control [70:30 methanol:water (v/v)]. The reaction mixture was kept for 4 min at room temperature (ca. 22 °C) before the absorbance at 593 nm was measured. A calibration curve was developed over a range of 0−500 μmol/L concentration of Trolox.

FT-IR Instrumentation and Spectral Collection. FT-IR spectra of garlic 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 to 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 respective 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°.

The extracts were allowed to equilibrate to room temperature (ca. 22 °C) before scanning. The methanolic aliquots of 20 μL each were uniformly spread directly onto the HATR crystal cell before spectra collection. Four aliquots were prepared from each garlic extract and tested in duplicate, for a total of eight spectra for each garlic sample (N = 25). 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 removes the interference of both methanol and water from the spectra and increases the intensity of the remaining bands. The same instrument 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).

Chemometric Analysis and Statistical Analysis. Infrared spectra were first preprocessed by EZ OMNIC 7.1a (Thermo Electron Inc., Lafayette, CO). Then, automatic baseline correction was employed 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 transforms using a nine-point Savitzky-Golay filter and wavelet transforms (with a scale of 7) were performed for spectral processing in Matlab (Math Works Inc., Natick, MA) to enhance the resolution of superimposed bands and to minimize problems from unavoidable baseline shifts.

Chemometric models were established based on processed spectra, including cluster analysis (principal component analysis, PCA), dendrogram

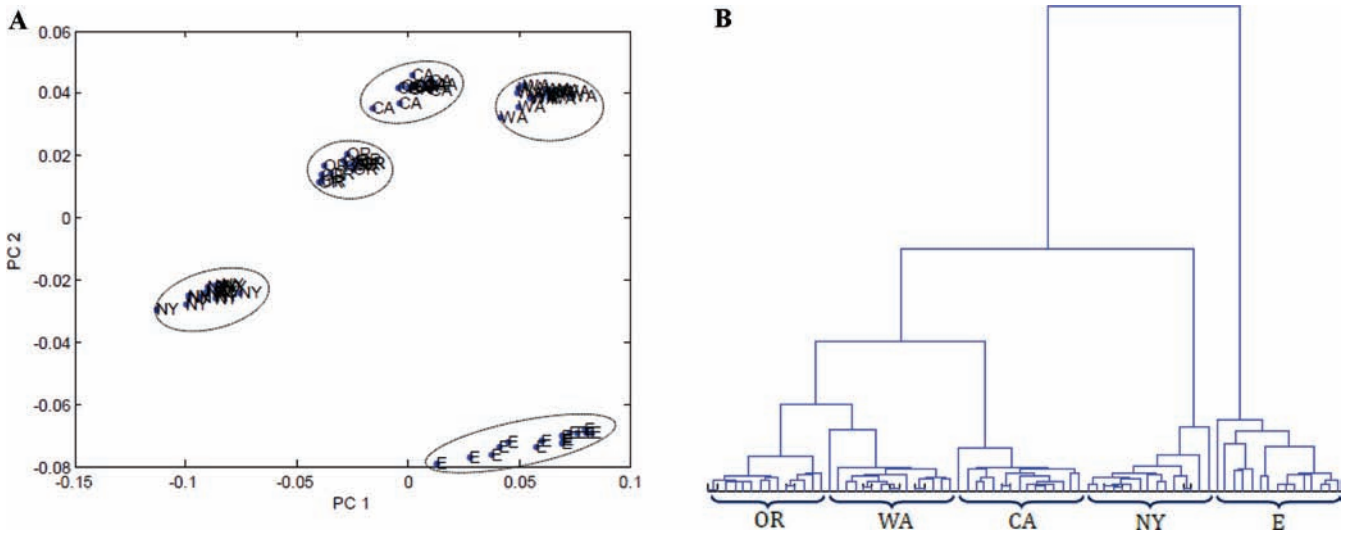

Figure 2. (A) Representative two-dimensional PCA results for elephant garlic (E) and garlic from different growing locations (NY, OR, CA, and WA). (B) Representative dendrogram analysis (DFA) results for elephant garlic (E) and garlic from different growing locations (NY, OR, CA, and WA).

Table 1. Summary of Measured TEAC, FRAP, DPPH, and TPC Reference Values of Garlic and Elephant Garlic Extracts[a]

	CA garlic	WA garlic	NY garlic	OR garlic	elephant garlic
TEAC (μmol Trolox/g FW)	57.86 ± 1.43 a	65.22 ± 3.32 b	63.24 ± 1.85 b	60.35 ± 4.44 b	47.50 ± 1.52 c
FRAP (μmol Trolox/g FW)	8.94 ± 0.31 a	11.45 ± 0.63 b	10.83 ± 0.15 b	10.86 ± 0.86 b	7.62 ± 0.64 c
DPPH (μmol Trolox/g FW)	7.60 ± 0.39 a	9.78 ± 0.37 b	9.47 ± 0.39 b	8.95 ± 0.44 b	6.95 ± 0.14 c
TPC (mg gallic acid/g FW)	15.61 ± 0.47 a	19.69 ± 0.61 b	17.35 ± 0.65 c	16.20 ± 0.42 a	15.62 ± 0.45 a

[a] ANOVA was used to compare data ($P < 0.05$); data sharing the same letter in a column (a−e) were not significantly different.

analysis (discriminant function analysis, DFA), partial least-squares regression (PLSR), and loading plot analysis. A minimum of 10 models were developed for each chemometric analysis. PCA is used to reduce the dimensionality of multivariate data while preserving most of the variances. Those selected unrelated principal components (PCs) are plotted and visualized in cluster forms.[16] DFA can construct branched dendrogram structures using prior knowledge of the composition of a biological sample.[7] PLSR was employed for quantitative analysis using Matlab. A total of eight spectra of each sample 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. The suitability of the developed models for predicting TPC and total antioxidant activity was assessed through a determination of regression coefficient (R), latent variables, standard error of calibration, and standard error of cross-validation.[7] Loading plots were derived from chemometric analyses and used for explaining segregation or linear regression of chemometric models based on various functional groups.[7] The wavenumbers between 1800 and 800 cm^{-1} were selected for all chemometric analyses in the current study.

The experiment was performed in three independent replicate trials. The results were expressed as the mean of three independent replicates ± standard deviations. The data were analyzed by one-way analysis of variance (ANOVA) and Student's t test to evaluate the significant difference at $P < 0.05$ using Matlab.

RESULTS AND DISCUSSION

FT-IR Spectral Features of Garlic and Elephant Garlic. The FT-IR spectral features of garlic are shown in Figure 1. The spectral features of the raw garlic samples were similar, and visual inspection was not enough to justify spectral variations (data not shown). The bands between the wavenumber of 1800 and 800 cm^{-1} reflected the chemical compositions of plant extracts, specifically lipids, proteins, polysaccharides, and polyphenols. The band at 1635 cm^{-1} is assigned to β-sheet structure of amide I,[17] while the band at 1403 cm^{-1} is assigned to symmetric CH_3 bending modes of the methyl groups of proteins.[17] The band at 1370 cm^{-1} is assigned to stretching C−O, deformation C−H, and deformation N−H.[17] The band at 1280 cm^{-1} is assigned to amide III band components of proteins.[3] The band at 1224 cm^{-1} is assigned to asymmetric stretching of phosphate groups of phosphodiester linkages in DNA and RNA.[17] The band at 1125 cm^{-1} is assigned to υ(CC) ring of polysaccharides and cellulose.[17] The distinctive band at 1025 cm^{-1} is assigned to vibrational frequency of $-CH_2OH$ groups of carbohydrates.[3] The band at 926 cm^{-1} is assigned to phosphodiester stretching bands.[17] The band at 813 cm^{-1} is assigned to ring CH deformation.[17] For high wavenumbers, the band at 3260 cm^{-1} is assigned to N−H stretching of proteins and O−H stretching of carbohydrates and water,[7] while the band at 2928 cm^{-1} is assigned to CH_2 antisymmetric stretch of methyl group mainly from lipids.[18]

Classification of Garlic and Elephant Garlic Samples by PCA and DFA. Unsupervised cluster analysis (PCA) and supervised dendrogram analysis (DFA) were performed to garlics according to FT-IR spectral features. First, PCA was employed to plot class projection based upon the first three PCs. The two-dimensional cluster segregation is shown in Figure 2A. The elephant garlic was well separated from the other four garlic

Table 2. PLSR Models (900−1800 cm^{-1}) for Determination of Total Antioxidant Activity in Garlic and Elephant Garlic ($N = 25$)

antioxidant Assay	antioxidant activity range (μmol Trolox/g FW)	no. of spectra	latent variables	R-Val	SECV[a] (μmol Trolox/g FW)	R-Cal	SEC[b] (μmol Trolox/g FW)
TEAC	45.66−68.93	200	6	0.97	1.45	0.98	1.33
FRAP	6.78−12.09	200	7	0.97	0.36	0.99	0.24
DPPH	6.76−10.21	200	6	0.96	0.33	0.97	0.19

[a] SECV, standard error of cross validation. [b] SEC, standard error of calibration.

Table 3. PLSR Model (2700−3600 and 900−1800 cm^{-1}) for Determination of TPC in Garlic and Elephant Garlic ($N = 25$)

TPC assay	TPC range (mg gallic acid/g FW)	no. of spectra	latent variables	R-Val	SECV (mg gallic acid/g FW)	R-Cal	SEC (mg gallic acid/g FW)
Folin−Ciocalteu	14.99−20.34	200	8	0.94	0.55	0.96	0.43

Table 4. PLSR Models for Predicted Antioxidant Activity in Garlic and Elephant Garlic Using FT-IR for the TEAC, FRAP, and DPPH Assays

antioxidant assay	sample	reference value (μmol Trolox/g FW)	SD	CV (%)	IR predicted value (μmol Trolox/g FW)	SD	CV (%)
TEAC	CA garlic	59.40	0.52	0.88	58.93	0.45	0.76
	WA garlic	63.15	0.20	0.32	63.56	0.36	0.57
	NY garlic	61.43	0.60	0.98	59.32	0.85	1.43
	OR garlic	57.91	0.59	1.02	56.23	0.55	0.98
	elephant garlic	48.64	0.46	0.95	49.32	0.77	1.56
FRAP	CA garlic	9.02	0.13	1.44	9.31	0.29	3.11
	WA garlic	11.87	0.12	1.01	11.35	0.32	2.82
	NY garlic	10.50	0.26	2.48	10.98	0.41	3.73
	OR garlic	10.12	0.10	0.99	10.26	0.28	2.73
	elephant garlic	7.42	0.20	2.70	7.57	0.49	6.47
DPPH	CA garlic	7.86	0.13	1.65	8.02	0.36	4.49
	WA garlic	9.60	0.10	1.04	9.65	0.19	1.97
	NY garlic	9.62	0.06	0.62	9.67	0.32	3.31
	OR garlic	8.75	0.09	1.03	8.91	0.69	7.74
	elephant garlic	6.97	0.06	0.86	6.92	0.25	3.61

Table 5. PLSR Model for Predicted TPC in Garlic and Elephant Garlic Using FT-IR for the Folin−Ciocalteu Assay

TPC	sample	reference value (mg gallic acid/g FW)	SD	CV (%)	IR predicted value (mg gallic acid/g FW)	SD	CV (%)
Folin−Ciocalteu	CA garlic	15.75	0.12	0.76	15.99	0.61	3.81
	WA garlic	19.02	0.05	0.26	20.11	0.39	1.94
	NY garlic	17.48	0.10	0.57	17.87	0.23	1.29
	OR garlic	15.81	0.07	0.44	15.32	0.17	1.11
	elephant garlic	15.63	0.19	1.22	15.04	0.55	3.66

clusters mainly by PC1 and the garlic from the four different growing states could also be clearly differentiated from each other forming tight clusters with interclass distances ranging from 5.96 to 29.12 based on Mahalanobis distance measurements computed between the centroids of classes. Clusters with interclass distance values higher than 3 are considered to be significantly different from each other.[7] In addition, a dendrogram-based chemometric model was derived based on selected PCs from PCA model using hierarchical cluster analysis (Figure 2B). Spectral features of elephant garlic and garlics from four different locations were distinctive with no sample misclassified ($n = 30$).

Quantitative Analysis of TPC and Antioxidant Activity of Garlic and Elephant Garlic. The antioxidant activity of garlics originated from four different locations was measured by three selective antioxidant assays, namely, TEAC, FRAP, and DPPH, while the TPC values were measured by Folin−Ciocalteu assay (Table 1). The FRAP values in extracts of garlic and elephant garlic were slightly higher than those obtained by the DPPH method ($P < 0.05$); however, they were significantly lower than those of the TEAC method ($P < 0.05$). The Folin−Ciocalteu method for determination of phenolic compounds is similar to antioxidant activity determination; therefore, the values should at least partially express antioxidant activity.[19] Tsai et al.[20] demonstrated that a significant correlation ($P < 0.05$) existed between antioxidant activity and TPCs. The variations of antioxidant activity measured by different chemical assays validated the problems of using a single one-dimensional method to evaluate

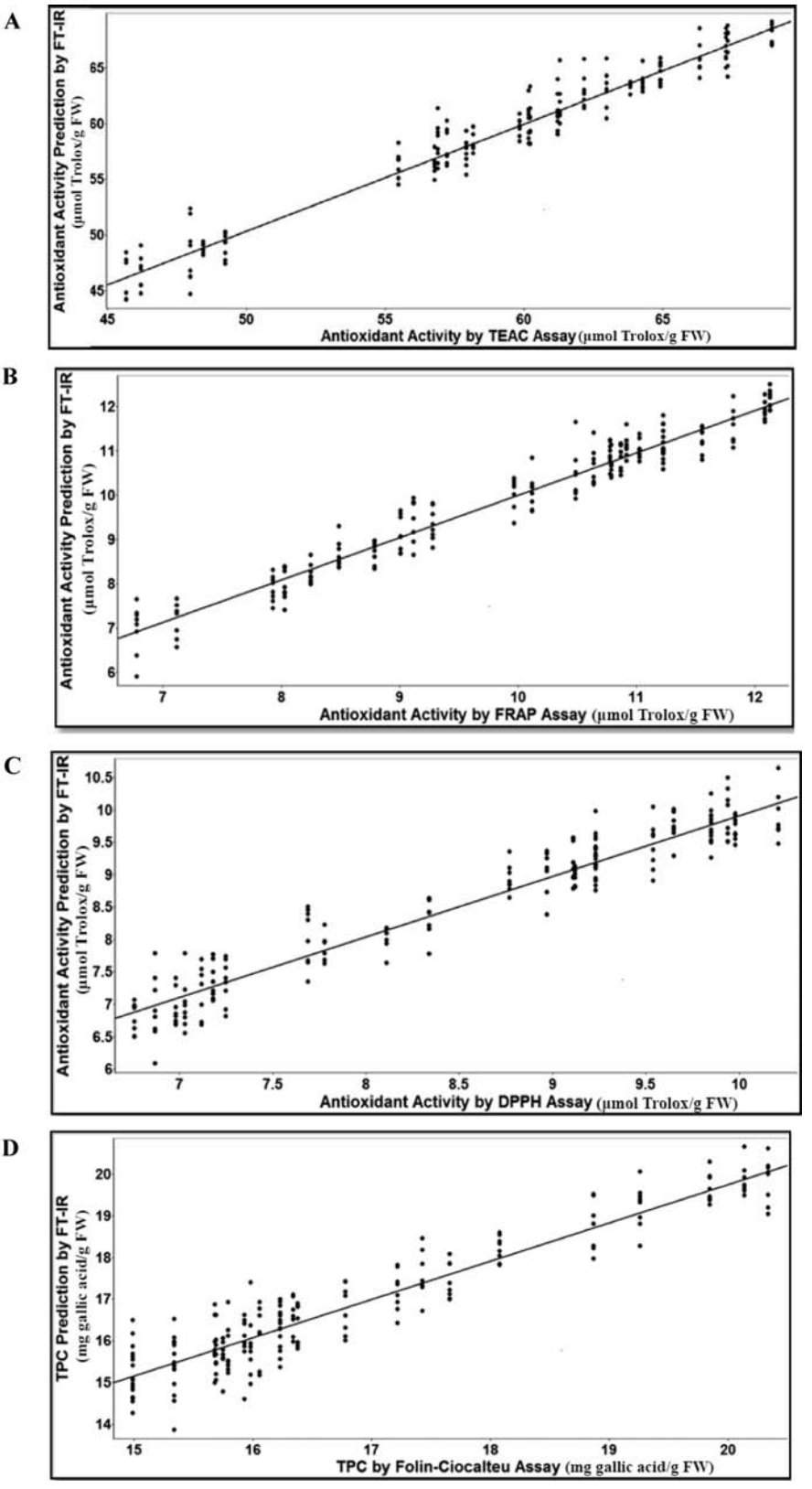

Figure 3. Cross-validated (leave-one-out) PLSR plots for antioxidant activity in garlic and elephant garlic using (A) TEAC assay, (B) FRAP assay, (C) DPPH assay, and (D) Folin−Ciocalteu assay.

multifunctional food and biological antioxidants.[15] The trend observed here for results of the three chemical assays for antioxidant activity determination was also observed in previous vegetable studies.[21] The TPC and antioxidant activity of elephant garlic are significantly lower than the other garlics ($P < 0.05$) (Table 1). The TPC and antioxidant activity values measured by

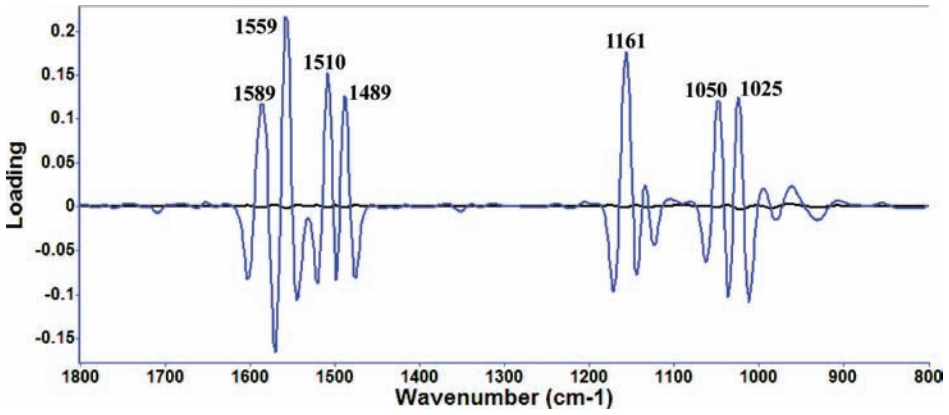

Figure 4. Representative loading plots of the first PC obtained from PLSR to explain antioxidant activity.

selective chemical assays in the current study were in the same range as previously reported.[1−3,22]

The reported antioxidant activities of garlic measured by selective chemical-based assays by different studies were in a wide range likely because of the differences in cultivation conditions.[2] The biosynthesis of phenolic compounds and organosulfur compounds is affected by different cultivation conditions, such as weather conditions, plant location, and harvest period. This may have explained at least some of the variation in antioxidant activities of garlics from different growing locations in current studies (Table 1). In addition, cultivar selection would also play a role.

The method of extracting bioactive components from plant materials is an important factor in measurements of antioxidant activity. For example, the recovery of phenols in extracts of vegetable matter is associated with the polarity of the solvents, and pure solvents result in low recovery of phenols as compared to aqueous organic solvents. Park and Chin[23] recently observed that methanol extracted garlic had a greater TPC, DPPH radical scavenging activity, and reducing power than a water extracted one, whereas the latter had a greater yield and iron chelating ability as compared to the former. Strail et al.[21] used an aqueous methanol (1:1, v/v) to extract polyphenols from various plants, including *Allium* species. Bozin et al.[1] used 80% methanolic solution to extract polyphenols from garlic. Frankel and Meyer[15] gave an extensive review to illustrate the problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. Therefore, in the current study, we used three chemical assays to quantify antioxidant activity and provided those as reference values for spectral chemometric analysis. In addition, a water−methanol extract hastened spectral measurements and provided greater resolution of spectral features as compared to the pure methanolic extract due to more uniform film formation (data not shown).

PLSR using the wavenumber from 1800 to 800 cm^{-1} was performed to establish linear regression between reference values (measured by chemical based assays) and FT-IR spectral features (Figure 3). Parameters of leave-one-out cross-validated PLSR models for TPC and antioxidant activity are summarized in Tables 2 and 3. A good PLSR model should have a high value for the regression coefficient ($R > 0.95$) and a low standard error of both calibrated and cross-validated models. In addition, a latent variable less than 10 is desired for PLSR model to avoid use of spectral noise as useful bands in the model, called "overfitting".[7,16] The standard error of calibration and standard error of cross-validation was less than 1.45, 0.36, and 0.33 μmol Trolox/g FW of extracts for TEAC, FRAP, and DPPH assays, respectively (Table 2), and 0.55 mg gallic acid/g FW of extracts for the Folin−Ciocalteu assay (Table 3). The models that we established and validated in the current study satisfied these requirements; thus, this study provides convincing results for both validation and further prediction. These validated PLSR models were employed to predict TPC and antioxidant activity of elephant garlic and garlics from selective locations for an independent set of samples ($N = 20$). Precision and errors of prediction results for the FT-IR PLSR models were comparable to those associated with the reference chemical-based assays (Tables 4 and 5).

The PLSR loading plots identified wavenumbers associated with the high contribution in the linear regression model. In other words, the band at a specific wavenumber results from vibrational properties of a definitive functional group, and this could be related to one or more chemical components in an analyte.[7,16] The PLSR models provided similar loading plots, and a representative one was selected and shown in Figure 4. The band at 1589 cm^{-1} is assigned to ring C−C stretch of phenyl.[3] The band at 1559 cm^{-1} is assigned to ring base.[17] The band at 1510 cm^{-1} is assigned to in-plane CH bending vibration from the phenyl ring.[17] The band at 1489 cm^{-1} is assigned to in-plane CH bending vibration.[18] The band at 1161 cm^{-1} is assigned to stretching vibrations of hydrogen-bonding C−OH groups.[17] The band at 1050 cm^{-1} is assigned to C−O stretching coupled with C−O bending of the C−OH of carbohydrates.[18] The band at 1025 cm^{-1} is assigned to vibrational frequency of $-CH_2OH$ groups of carbohydrates.[3] The loading plots analysis in the current study support previous studies that phenyl structure and hydroxyl functional groups are tightly related to antioxidant activity of fruits and vegetables.[21] Both Queiroz et al.[2] and Bozin et al.[1] observed a strong relationship between phenolic content and antioxidant activity of fresh garlic. In other studies, thiosulfinates (mainly allicin and ajoene),[4,24] diallyl sulfides,[5] other *S*-alk(en)yl-L-cysteine sulfoxides,[4] and recently sulfenic acids[25] were found to contribute significantly to antioxidant activity of fresh garlic. The SO_2 and SO groups of sulfur compounds produce infrared bands in the 1400−1000 cm^{-1} range. However, the concentration of small volatile compounds in biological samples could not be determined due to matrix interference;[17] other sulfur containing bonds, such as S−H, S−S, and C−S, produce weaker infrared bands. Compounds, such as sulfides containing C−S and S−S bonds, show stretching bands at

700−600 cm^{-1} and near 500 cm^{-1}, respectively, in simple systems such as water or air, but spectral features have not been reported for more complex matrices. The weak S−H stretching band appears near 2500 cm^{-1}.[26] Similarly, spectral features for these sulfur-containing compounds could not be detected by us in this study in chemically complex plant tissue extracts (Figure 1). In the current study, we show that phenolics and hydroxyl functional groups contributed significantly to the antioxidant activity of fresh garlics on the basis of loading plot studies in the PLSR model but were unable to directly determine the contribution of the sulfur containing antioxidants.

In conclusion, infrared spectroscopy coupled with chemometrics could be employed to quantify and predict antioxidant activity and TPC of garlic and elephant garlic with a precision similar to that obtained by chemical assays. Furthermore, spectroscopic segregation models (PCA and DFA) could differentiate garlics originating from different growing locations. In the future, this rapid detection method may have potential to be used to determine antioxidant activity of other plant materials.

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