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Analytical Methods

Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy

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ABSTRACT

Total phenolic content (TPC) and total antioxidant capacity (TAC) of four onion varieties (red, white, yellow and sweet) and shallot from selected locations (Washington, Idaho, Oregon, Texas and Georgia) were determined using Fourier transform infrared (FT-IR) spectroscopy (4000–400 cm^{-1}). The Folin–Ciocalteu (F–C) assay was used to quantify TPC and three assays were used to determine TAC, including 2,2-diphenyl-picrylhydrazyl (DPPH) assay, Trolox equivalent antioxidant capacity (TEAC) assay and ferric reducing antioxidant power (FRAP) assay. Partial least squares regression (PLSR) with cross-validation (leave-one-out) was conducted on onion and shallot extracts ($n = 200$) and their corresponding F–C, DPPH, TEAC and FRAP values were employed to obtain four independent calibration models for predicting TPC and TAC for the extracts. Spectra from an extra 19 independent extracts were used as an external validation set for prediction. A correlation of $r > 0.95$ was obtained between FT-IR predicted and reference values (by F–C, DPPH, TEAC and FRAP assay) with standard errors of calibration (SEC) and standard errors of cross-validation (SECV) less than 2.85, 0.35 and 0.45 μmol Trolox/g FW of extracts for TEAC, FRAP and DPPH assay, respectively; and 0.36 mg gallic acid/g FW of extracts for the F–C assay. In addition, cluster analysis (principal component analysis (PCA)) and discriminant function analysis (DFA) could differentiate varieties of onions and shallot based upon infrared spectral features. Loading plots for the various chemometrics models indicated that hydroxyl and phenolic functional groups were most closely correlated with antioxidant capacity. The use of mid-infrared spectroscopy to predict the total antioxidant capacity of vegetables provides a rapid and precise alternative to traditional wet chemistry analysis.

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1. Introduction

Consuming vegetables and fruits may reduce the risk of chronic diseases, including cardiovascular disease, stroke, neurodegeneration, and type II diabetes. Substantial recent research has been performed to investigate the potential health benefits of antioxidants in food. Antioxidants can inhibit oxidative reactions *in vivo*, and aid in functional performance of enzyme systems for self-defence mechanisms within cells (Lee, Koo, & Min, 2004). Among all vegetables, onion is a species consumed widely across the world and possesses a high content of flavonoid compounds (mainly quercetin and its conjugates) and sulphur compounds (i.e. thiosulphinates), both of which have a high level of antioxidant activity (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002).

Antioxidants can deactivate radicals by three major mechanisms: hydrogen atom transfer (HAT), electron transfer (ET) and combination of both HAT and ET (Prior, Wu, & Schaich, 2005). HAT measures the ability of an antioxidant to quench free radicals by hydrogen donation. ET detects the ability of antioxidant to transfer one electron to reduce radicals, metals and carbonyls (Huang, Ou, & Prior, 2005). FRAP is an ET assay and the F–C assay measures the TPC using an ET mechanism. Oxygen radical absorbance capacity (ORAC) assay is a common HAT based competitive assay. TEAC and DPPH assays combine both HAT and ET mechanisms.

The TPC and TAC of vegetables have been studied extensively using the various antioxidant assays mentioned above (Stratil, Klejdus, & Kuban, 2006). However, 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 screened. Infrared spectroscopy (IR) provides a unique advantage of simple sample preparation

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while retaining satisfactory precision and sensitivity (Movasaghi, Rehman, & Rehman, 2008; Naumann, 2001). It is gaining wider use in food analysis and in non-destructive applications (Thygesen, Lokke, Micklander, & Engelsen, 2003). Lu and Rasco (in press) compiled a comprehensive review of mid and near-IR determination of antioxidant content and activity and the relevant chemometrics. Quantifying TAC in foods by IR is a new application. Near-infrared spectroscopy (NIR) has been used to predict TAC in green tea using PLSR model with TEAC reference values (Zhang, Luypaert, Fernandez Pierna, Xu, & Massart, 2004). In another study, flavonoid content, TPC and TAC of rice grain were studied using NIR with PCA, PLSR, and modified PLSR. TAC of red wine was quantified by mid-IR (FT-IR) using PLSR (Versari, Parpinella, & Rio, 2010) and TAC of fruit extracts using a similar method (Lam, Proctor, Howard, & Cho, 2005). In this study, methanol–water–formic acid (60:37:3, v/v/v) was used to extract fruits and ORAC assay was employed as the reference method. To date, there is no reported research work on quantifying TAC and TPC in vegetables by IR with limited studies on predictions of antioxidant concentration and investigation specific functional groups involved in explaining TAC and TPC activity in the relevant wavenumber regions. This is advantage of a unique feature of bioanalytical spectroscopy compared with other forms of instrumental analysis. Thus, the objectives of this study are to: (1) quantify TPC and TAC in onion and shallot by FT-IR; and (2) investigate which functional groups are the primary contributors to TPC and TAC providing a new approach to explain the free radical quenching mechanism of antioxidants in *Allium* sp. IR may provide a rapid alternative method for food producers and consumers, increasing the available information about the concentration and potential biological activity of various foods and the biological variability in antioxidant activity resulting from cultivar, cultivation and storage conditions.

2. Materials and methods

2.1. Chemicals and reagents

Folin–Ciocalteu reagent, 2,2-diphenyl-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonate) 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 gallic acid were obtained from Sigma–Aldrich (St. Louis, MO, USA). Sodium carbonate (Na_2CO_3), methanol, potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), sodium hydroxide and hydrochloric acid were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). All reagents and solvents used were analytical or HPLC grade.

2.2. Preparation of onion samples

Four onion varieties, namely red onion, white onion, yellow onion and sweet onion and shallot (originated from the States of Washington, Idaho, Texas, California and Georgia, USA) were purchased from local grocery stores twice. All plants were from the 2009 crop year and no older than 5 months. Material was selected that was free from visible blemishes or defects. Onions and shallots were stored in the dark at 4 °C until analysis within 2 weeks of purchase. The outer layers of the onions were removed manually, and the remaining tissues pureed in a PowerBlend Duet™ blender/food processor (Cuisinart, NJ, USA). The puree was freeze-dried. The freeze-dried onion was ground in a mortar to a fine powder which was then stored at 4 °C in amber vials in a dry atmosphere. All data collected for each onion variety were reported as mean ± standard deviation (SD) for at least three replicate experiments.

2.3. Extraction of polyphenols

Aqueous methanol is one of the most commonly used solvents for the extraction of flavonoids present in onions and other vegetables. The extraction method used here is based upon that of Santas, Carbo, Gordon, and Almajano (2008) with minor modifications. Thirty millilitres of methanol:water (70:30 v/v) was added to freeze-dried onion powder (3 g). After 30 min of extraction with magnetic stirring at 900 rpm at room temperature (ca. 20 °C), the extract was centrifuged at 3000 rpm (accu Spin™ 400, Fisher Scientific, Pittsburgh, PA, USA) for 15 min. Supernatant was recovered and extraction repeated twice more for 45 and 90 min. Finally, the three supernatants were pooled and stored at 4 °C in the dark. Extractions were performed in triplicate and light exposure was avoided during the extraction process.

2.4. Determination of TPC

Total phenolic content of each extract was determined in duplicate by the Folin–Ciocalteu procedures according to the method of Sun, Powers and Tang (2007) and modified by Sun, Powers, and Tang (2007) with minor changes. In brief, Folin–Ciocalteu reagent was diluted 10-fold with deionised water. The 70% methanolic onion or shallot 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. 20 °C). Then, 0.75 mL of 2% sodium carbonate (w/v) solution was added. The mixture was allowed to stand in the dark (ca. 20 °C) for 45 min before measuring the absorbance at 765 nm using an Ultrospec 4000 UV–Visible spectrophotometer (Pharmacia Biotech, Cambridge, UK) against a blank, containing deionised 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).

2.5. Determination of TAC

2.5.1. DPPH Assay

The antioxidant capacity of the onion and shallot extracts was measured using a DPPH method described by Sun, Tang, and Powers (2005) and Cheng, Moore, and Yu (2006) using the free radical 2,2-diphyenyl-picrylhydrazyl (DPPH), with some 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. 20 °C) (Cheng et al., 2006). Seventy percent methanolic solutions of Trolox in a range of 0–500 μmol/L were used for calibration to compare the antioxidant capacity of onion and shallot extracts. The antioxidant capacity of the sample was expressed as μmol Trolox equivalents/g fresh weight sample (μmol Trolox/g FW).

2.5.2. TEAC Assay

This assay is based on the decolourisation of the radical cation 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonate) $ABTS^+$ after reduction to ABTS. Spectrophotometric methods were performed as described by Schilling et al. (2007), Maier, Schieber, Kammerer, and Carle (2009) and van den Berg, Haenen, van den Berg, and Bast (1999). 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 sodium chloride. For the daily preparation of the ABTS solution, 0.5 mL 20 mmol/L ABTS in the phosphate buffer was mixed with 100 mL of 2,2′-azobis(2-amidinopropane)dihydrochloride (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 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). Absorbance was then measured at 734 nm (van den Berg, Haenen, van den Berg, van der Vijgh, & Bast, 2000). 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).

2.5.3. FRAP assay

This method is based on the increase in absorbance at 593 nm due to the formation of tri-pyridyl-S-triazine complexes with Fe^{2+} [TPTZ-Fe(II)] in the presence of a reducing agent (Benzie & Strain, 1996; Benzie & Szeto, 1999). The FRAP reagent was prepared from 2.5 mL of a TPTZ solution (10 mmol/L) in hydrochloric acid (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 deionised water and 100 μL of the sample extracts, Trolox standard or control (methanol:water). The reaction mixture was allowed to stand for 4 min at room temperature (ca. 20 °C) before the absorbance at 593 nm was measured. A calibration curve was performed over a range of 0–500 μmol/L concentration of Trolox.

2.6. FT-IR instrumentation and spectral collection

FT-IR spectra of onion and shallot extracts were recorded at room temperature (ca. 20 °C) using a Nicolet Avatar 380 spectrometer (Thermo Electron Inc., San Jose, CA, USA) 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, USA). Spectrum of each sample was an average of 128 respective scans with two spectra taken per aliquot (Al-Qadiri, Al-Alami, Al-Holy, & Rasco, 2008). 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. 20 °C) before scanning. The methanolic aliquots of 20 μL each were uniformly spread directly onto the HATR crystal cell before spectra collection. The aliquots were dried to form a uniform layer on the surface of crystal cell of infrared spectrometer under room temperature (ca. 20 °C) within ~5 min. Drying the sample into a film removes the interference of methanol and water from the spectra and increases intensity of spectral bands (Lu & Rasco, 2010). 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, USA).

2.7. Data processing and chemometrics

The Matlab & Simulink R2010a programs (The MathWorks, Inc., Natick, MA, USA) were used to perform cluster analysis and dendrogram analysis while DeLight 3.2.1 (Textron Systems, Wilmington, MA, USA) was used for linear regression analysis. The loading plots for the chemometric models were examined using both Matlab & Simulink and DeLight software.

2.7.1. Data processing

Data preprocessing was conducted before chemometric model development. This included automatic baseline correction to flatten the baseline and normalisation to compensate for the path-length differences resulting from differences in sample thickness, both of which make comparison of spectral features possible (Lu et al., 2010). Other spectral treatments for optimisation of results were followed, including binning (2 cm^{-1}), smoothing (Gaussian function over 9 cm^{-1}), and second derivative transformation with a gap value of 12 cm^{-1}. The data preprocessing was performed in OMNIC and Matlab & Simulink.

2.7.2. Chemometric model development

The infrared spectrum consists of many related variables (wavenumber) which are difficult to analyse. The purpose of chemometrics is to simplify or dimensionally reduce the data set to fewer independent parameters, with minimal loss of information, thereby making human interpretation possible (Goodacre, 2003). PCA is a well-known technique for reducing the dimensionality of multivariate data while preserving most of the variances, and this technique is used to identify correlations among a set of variables and to transform the original set of variables to a new set of uncorrelated ones called principal components (PCs). These PCs are then plotted and clusters in the data visualised (Lu et al., 2010). DFA is another form of chemometrics which can be used to construct branched dendrogram structures (Jarvis & Goodacre, 2004). PLSR is a bilinear regressed analytical method that establishes the relationship between spectral features and reference values (i.e. concentration of analytes). PLSR models were evaluated in terms of latent variables, correlation coefficient (r value), standard error and outlier diagnostics. The calibration PLSR model is created, following by cross validation (either leave-one-out or from other batches of data). The validated calibration model could be performed to do prediction of samples outside. The selection of optimum latent variables is critical to model performance (Alsberg, Kell, & Goodacre, 1998). The wavenumbers between 1800 and 900 cm^{-1} were selected for the establishment of all chemometric models (PCA, DFA and PLSR) in current study.

2.8. Statistical analysis

Each experiment was performed in triplicate. The average values and standard deviations were calculated using Excel (Microsoft Inc., Redmond, WA, USA). The data were analysed by one-way analysis of variance (ANOVA) and t-test to evaluate the significant difference at $P < 0.05$ using Matlab & Simulink.

3. Results and discussion

3.1. FT-IR spectral features of onions and shallot

The spectral features of onions and shallot were shown in Fig. 1. The bands between the wavenumbers of 1800–750 cm^{-1} (fingerprint regions) reflected the biochemical compositions, especially the moieties of carbohydrate, lipid, protein secondary structures (α-helix, β-sheet and random coil), and polyphenols in plants. The distinctive peak at the wavenumber of 1618 cm^{-1} is assigned to ring C—C stretch of phenyl (Schulz & Baranska, 2007), which is present at high levels in the polyphenolic components in *Allium* plants. The band at 1405 cm^{-1} is due to CH_3 asymmetric deformation (Agarwal, Tandon, & Gupta, 2006). The peak at 1339 cm^{-1} is due to the in-plane C—O stretching vibration combined with the ring stretch of phenyl (Schulz & Baranska, 2007). The minor band at 1255 cm^{-1} is from amide III (random coil) for protein (Chiriboga, Xie, Vigorita, Zarou, & Diem, 1998). The wavenumber region between 1200 and 950 cm^{-1} contains functional groups mainly from carbohydrate. The "shoulder" peak at 1105 cm^{-1} (Andrus, 2006) is from carbohydrate while the bands at 1025 and 985 cm^{-1} are due to vibrational frequency of —CH_2OH groups of carbohydrates (Mordechai et al., 2001) and OCH_3 from polysaccharides-cellulose (Shetty, Kedall, Shepherd, Stone, & Barr, 2006), respectively. The

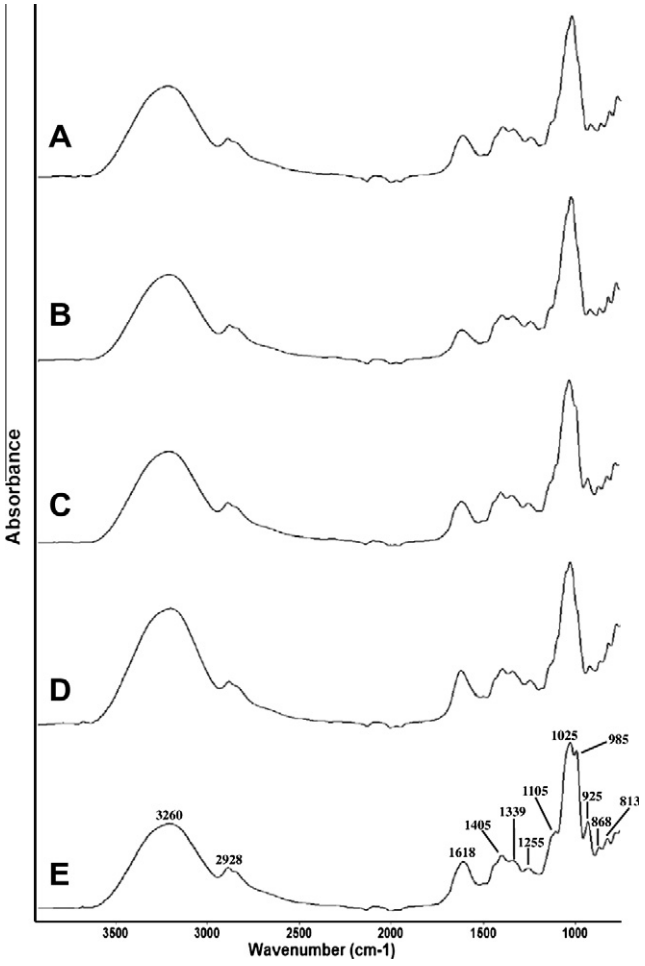

Fig. 1. Representative FT-IR raw spectra of white onion (A), yellow onion (B), red onion (C), sweet onion (D) and shallot (E).

bands at both 925 and 868 cm^{-1} are assigned to the left-handed helix DNA (Z form) (Dovbeshko et al., 2002). The band at 813 cm^{-1} is caused by ring CH deformation (Schulz & Baranska, 2007), which could also reflect structural information about polyphenols. For the higher wavenumbers (lower frequencies), the peak at 3260 cm^{-1} is due to N—H stretching of proteins and O—H stretching of carbohydrates and water while the peak at 2928 cm^{-1} is due to CH_2 antisymmetric stretch of methyl groups mainly from lipids (Lu & Rasco, 2010). The raw spectral features of four onion varieties were similar while shallot presented a clear different spectral feature around the wavenumber of 1000 cm^{-1}.

3.2. Classification of onion and shallot samples by PCA and DFA analysis

Cluster and dendrogram analyses were developed simultaneously to sort different onion varieties and shallot according to FT-IR spectral features between the wavenumber regions of 1800–900 cm^{-1} using Matlab & Simulink. First, PCA-DF was employed to plot class projection based on the first five PCs. The three-dimensional cluster segregation is shown in Fig. 2(a). The "shallot cluster" was well separated from four "onion clusters" and even the four "onion clusters" were also clearly separated and tightly clustered with interclass distances ranging from 4.2 to 34.5 based upon *Mahalanobis distance* measurements computed between the centroids of classes. Clusters with interclass distance values higher than 3 are believed to be significantly different from each other (De Maesschalck, Candofi, Massart, & Heuerding, 1999). From this information, a composite dendrogram was derived using hierarchical cluster analysis and was established based on the PC-DFA space and from the selected PCs from the PCA model (Fig. 2(b)). Spectral features of shallot and four onion varieties were distinctive with no samples misclassified ($n = 50$).

The loading plots of the first 3 PCs obtained from PC-DFA explained the segregation obtained in this study (Fig. 3(a)). The first principal component composed more than 80% of the segregation. The bands positively associated with the most discrimination among different varieties of onions and shallots are located around the wavenumber of 1000 cm^{-1} and between the wavenumbers from 1800 to 1500 cm^{-1}. Most of these bands are associated with phenolic ring structures. For example, the distinctive band at the wavenumber of 1630 cm^{-1} is from the ring C—C stretch of phenyl (Schulz & Baranska, 2007); the band at the wavenumber of 1559 cm^{-1} is due to the ring base (Dovbeshko, Gridina, Kruglova, & Pashchuk, 1997); the band at the wavenumber of 1510 cm^{-1} is assigned to in-plane CH bending vibration from the phenyl rings (benzene) (Schulz & Baranska, 2007). Other bands are assigned to the presence of carbohydrates. For example, the band at the wavenumber of 1078 cm^{-1} is from the C—OH stretching band of oligosaccharide residue (Yoshida et al., 1997); the band at the wavenumber of 1030 and 972 cm^{-1} are due to glycogen vibration (CH_2OH vibration) (Wang, Wang, & Huang, 1997) and OCH_3 (polysaccharides and pectin), respectively (Shetty et al., 2006). The band at the wavenumber of 1679 cm^{-1} is assigned to the stretching C=O vibrations that are H-bonded (Dovbeshko et al., 1997) and the band at the wavenumber of 1736 cm^{-1} is assigned to the C=O stretching of lipids (Fabian et al., 1995). The loading plots of PC-DFA provided reasonable explanations that concentration of phenolic compounds and carbohydrate moieties (OH bond from carbohydrate) constitute the greatest variation in antioxidant extracts between different types of onions and shallot. In addition, the PC-DFA system (both cluster analysis and dendrogram analysis) could not provide satisfactory sorting results for onions of the same variety (i.e. red onion) from different production locations. This indicated that the differences among individual onions of the same type but from different harvest locations are less significant than the differences between different types of onions.

3.3. Quantitative analysis of TPC and TAC of onion and shallot

The TAC of four varieties of onions and shallot originated from five different locations were determined by three different antioxidant assays, namely TEAC, DPPH and FRAP with the TPC value determined by the F–C assay (Table 1). The FRAP values in extracts of onion and shallot 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 F–C method for the determination of phenolic compounds is similar to antioxidant capacity determination; hence, the values should at least partially express antioxidant capacity. The variations of TAC measured by different chemical assays validated the problems of using one-dimensional method to evaluate multifunctional food and biological antioxidants (Frankel & Meyer, 2000). The trend observed here for results of the three chemical assays for TAC determination was also observed by Stratil et al. (2006) for many types of vegetables, including yellow onion and red onion. The TPC and TAC value of shallot is significantly higher than the four varieties of onions ($P < 0.05$) (Table 1), which was similar to previous studies (Leelarungrayub, Rattanapanone, Chanarat, & Gebicki, 2006). The TPC and TAC values by various chemical assays obtained from the present study were in the same range as previously reported (Nuutila, Puupponen-Pimia, Aarni, & Oksman-Caldentey, 2003; Prakash, Singh, & Upadhyay, 2007; Roldan-Marin, Sanchez-

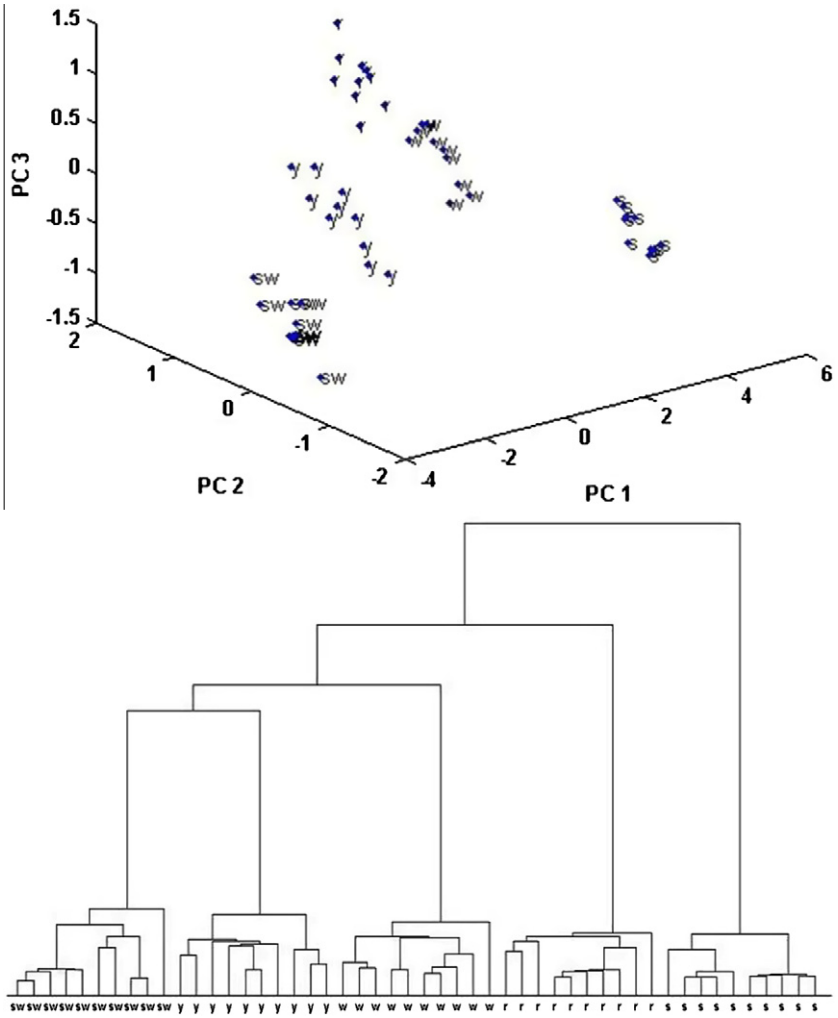

Fig. 2. (a) PC-DFA plot showing clusters obtained from the analysis of different onion species (*Allium cepa*) and shallot (*Allium oschaninii*); (b) a composite dendrogram derived from hierarchical cluster analysis utilising the PC-DFA space showing differentiation of onions and shallots by type. In this figure, r: red onion, y: yellow onion, sw: sweet onion, w: white onion and s: shallot.

Table 1
Summary of measured TEAC, FRAP, DPPH and TPC reference values of different onion varieties (*Allium cepa*) and shallot (*Allium oschaninii*) extracts.[a]

	White onion	Yellow onion	Red onion	Sweet onion	Shallot
TEAC (μmol Trolox/g FW)	11.82 ± 2.16a	15.22 ± 2.36b	28.18 ± 4.59c	10.56 ± 1.15a	34.40 ± 3.25d
FRAP (μmol Trolox/g FW)	4.38 ± 0.40a	5.32 ± 0.59b	5.76 ± 0.47b	2.48 ± 0.19c	6.40 ± 0.40d
DPPH (μmol Trolox/g FW)	3.04 ± 0.18a	4.56 ± 0.40b	5.20 ± 0.28c	1.42 ± 0.13d	5.71 ± 0.44e
TPC (mg gallic acid/g FW)	2.69 ± 0.22a	1.64 ± 0.14b	4.28 ± 0.28c	1.42 ± 0.08d	17.18 ± 1.88e

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

Moreno, Lloria, De Ancos, & Cano, 2009; Santas et al., 2008; Sellappan & Akoh, 2002; Stratil et al., 2006). However, the TAC and TPC values in the present study were lower than in some others, which included the skin (peel) in the analysis (Singh et al., 2009). It was well documented that the outer layers of *Allium* bulbs have the highest contents of TPC and TAC (mainly contributed by anthocyanins) followed by continuously decreasing levels towards the inner edible part of the bulb (Prakash et al., 2007).

The reported antioxidant capacity of onion measured by selective assays (i.e. DPPH, TEAC, FRAP) by different studies were in a wide range because of the differences in cultivation conditions (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). The biosynthesis of flavonoids was mainly affected by different cultivation conditions, such as weather conditions, plant location and harvest period. The content of flavonoids will directly affect the TPC and TAC values of samples. Genetics also play a role (Bilyk, Cooper, & Sapers, 1984; Galmarini, Goldman, & Harvey, 2001). The method of extracting polyphenols from plant materials is an important factor for determination of antioxidant activity. For example, the recovery of phenols in extracts of vegetable matter is associated with the polarity of the solvent (Santas et al., 2008) and pure solvents generally result in lower recovery of phenols compared to aqueous organic solvents.

The results (parameters) of cross validated (leave-one-out) PLSR models for TAC and TPC were shown in Table 2. These models were rigorous and based upon model parameters (spectral numbers, latent variable, correlation coefficient and standard error) and challenge data. The estimated values of TAC and TPC by FT-IR

Table 2
PLSR models (1800–900 cm^{-1}) for determination of total antioxidant activity and total phenolic content in onion (*Allium cepa*) and shallot (*Allium oschaninii*).[a]

Assay	Range	No. of samples	Latent variables	R-Val	SECV	R-Cal	SEC
TEAC	9.10–38.4	200	7	0.96	2.62	0.99	2.85
FRAP	2.20–6.90	200	6	0.97	0.35	0.99	0.31
DPPH	1.26–6.23	200	6	0.96	0.45	0.98	0.39
Folin–Ciocalteu	1.41–4.67	160	7	0.95	0.36	0.97	0.19

[a] For TEAC, FRAP and DPPH, the unit is μmol Trolox/g FW; for Folin–Ciocalteu, the unit is mg gallic acid/g FW.

spectroscopy gave good correlation coefficients ($r > 0.95$) with the measured reference values by TEAC, FRAP, DPPH and F–C assays. The standard error of calibration (SEC) and standard error of cross validation (SECV) was less than 2.85, 0.35 and 0.45 μmol Trolox/g FW of extracts for TEAC, FRAP, and DPPH assay, respectively and 0.36 mg gallic acid/g FW of extracts for F–C assay (Table 2). These PLSR models were used to predict TAC and TPC of onions and shallot for an independent set of samples ($n = 19$). Precision and errors of prediction results for the FT-IR models were comparable to those associated with the reference chemical assays (Table 3).

The PLSR loading plots identified wavenumbers associated with the highest variation (contribution) in the linear regression calibration model. The band at a specific wavenumber results from vibrational properties of a definitive functional group, and thus can be related to one or more chemical components in an analyte (Lu & Rasco, 2010). The PLSR models developed here provided similar loading plots and a representative one was selected and shown in Fig. 3(b). The first five latent variables explained most of the variance (>90%) in the PLSR model for determination of TPC and TAC. Major loading plots in first principal component are located at wavenumber of 1633 cm^{-1} and around the wavenumbers from 1200 to 900 cm^{-1} (Fig. 3(b)). Bands that significantly contribute to loading plots appeared as primary factors in latent variable analysis (De Nardso, Shiroma-Kian, Halim, Francis, & Rodriguez-Saona, 2009). The band at the wavenumber of 1633 cm^{-1} is assigned to ring C—C stretch of phenyl (Schulz & Baranska, 2007). The bands at the wavenumbers of 1160, 1137, 1050 and 1025 cm^{-1} are related to stretching vibrations of hydrogen-bonding C—OH groups (Wang et al., 1997), oligosaccharide C—OH stretching band (Yoshida et al., 1997), C—O stretching coupled with C—O bending of the C—OH of carbohydrates (Wang et al., 1997) and vibrational frequency of CH_2OH groups of carbohydrates (including glucose, fructose, etc.) (Huleihel et al., 2002; Mordechai et al., 2001), all of which are associated with hydroxyl functional group. This result supports previous studies associating phenyl structure and hydroxyl functional group with antioxidant capacity. Higher phenol content was associated with higher antioxidant capacity (Santas et al., 2008) and this was also validated in the case of onion (Rice-Evans, Miller, & Paganga, 1996). Even though the thiosulphinates and *S*-alk(en)yl-L-cysteine sulphoxides can contribute to antioxidant capacity (Shon, Choi, Kahng, Nam, & Sung, 2004; Xiao & Parkin, 2002), the flavonoids affect antioxidant capacity to a greater extent, especially quercetin and its glycoside conjugates (Yang, Meyers, Van der Heide, & Liu, 2004). The mechanism of action of quercetin includes chelation of transition metal ions, free radical scavenging and inhibition of oxidation (Lanzotti, 2006). Zielinska, Wiczkowski, and Piskula (2008) indicating that the antioxidant capacities of quercetin glucosides are most affected by a free hydroxyl group forming a catecholic set in ring B of these compounds. The structure features and nature of substitutions on ring B and C determined the active radical scavenging capacity of flavonoids. The finding of structure–activity relationship of flavonoids and phenolic acids was confirmed by Balasundram, Sundram, and Samman (2006) and in a review by Rice-Evans et al. (1996). Thus, the degree of hydroxylation and positions of hydroxyl groups in the ring structure have a substantial impact on the antioxidant capacity of onion, which we have validated here.

On the whole, FT-IR can be used to predict the antioxidant capacity of various onions and shallots with a precision similar to that obtained by conventional chemical assays. This method is simple and rapid after tissue extracts have been prepared. Multivariate analysis methods can also differentiate various *Alliums*

Table 3
PLSR models for predicted total antioxidant activity and total phenolic content in onion (*Allium cepa*) and shallot (*Allium oschaninii*) using FT-IR for TEAC, FRAP, DPPH and Folin–Ciocalteu assays.[a]

Assay	Sample	Reference value	SD	CV (%)	IR predicted value	SD	CV (%)
TEAC	White onion A (CA)	13.07	0.15	1.15	12.81	0.13	1.01
	Yellow onion A (GA)	16.68	0.53	3.18	17.98	0.72	4.00
	Red onion A (CA)	25.99	0.46	1.77	25.37	0.52	2.05
	Sweet onion A (WA)	11.29	0.34	3.01	11.35	0.19	1.67
	Shallot A (CA)	38.98	0.45	1.15	39.55	0.56	1.42
FRAP	White onion B (ID)	4.15	0.07	1.69	4.21	0.12	2.85
	Yellow onion B (CA)	4.94	0.10	2.02	5.11	0.24	4.70
	Red onion B (WA)	5.87	0.04	0.68	5.82	0.18	3.09
	Sweet onion B (TX)	2.71	0.06	2.21	2.78	0.11	3.96
	Shallot B (GA)	6.28	0.14	2.23	6.43	0.23	3.58
DPPH	White onion C (WA)	3.11	0.03	0.96	3.09	0.08	2.59
	Yellow onion C (WA)	4.78	0.06	1.26	4.72	0.13	2.75
	Red onion C (GA)	5.51	0.28	5.08	5.76	0.21	3.65
	Sweet onion C (CA)	1.33	0.04	3.01	1.38	0.09	6.52
	Shallot C (TX)	5.73	0.15	1.15	5.76	0.23	1.01
Folin–Ciocalteu	White onion D (TX)	2.65	0.21	7.92	2.62	0.19	7.25
	Yellow onion D (GA)	1.75	0.03	1.71	1.79	0.06	3.35
	Red onion D (WA)	4.54	0.04	0.88	4.52	0.11	2.43
	Sweet onion D (WA)	1.35	0.02	1.48	1.29	0.07	5.43

[a] For TEAC, FRAP and DPPH, the unit is μmol Trolox/g FW; for Folin–Ciocalteu, the unit is mg gallic acid/g FW.

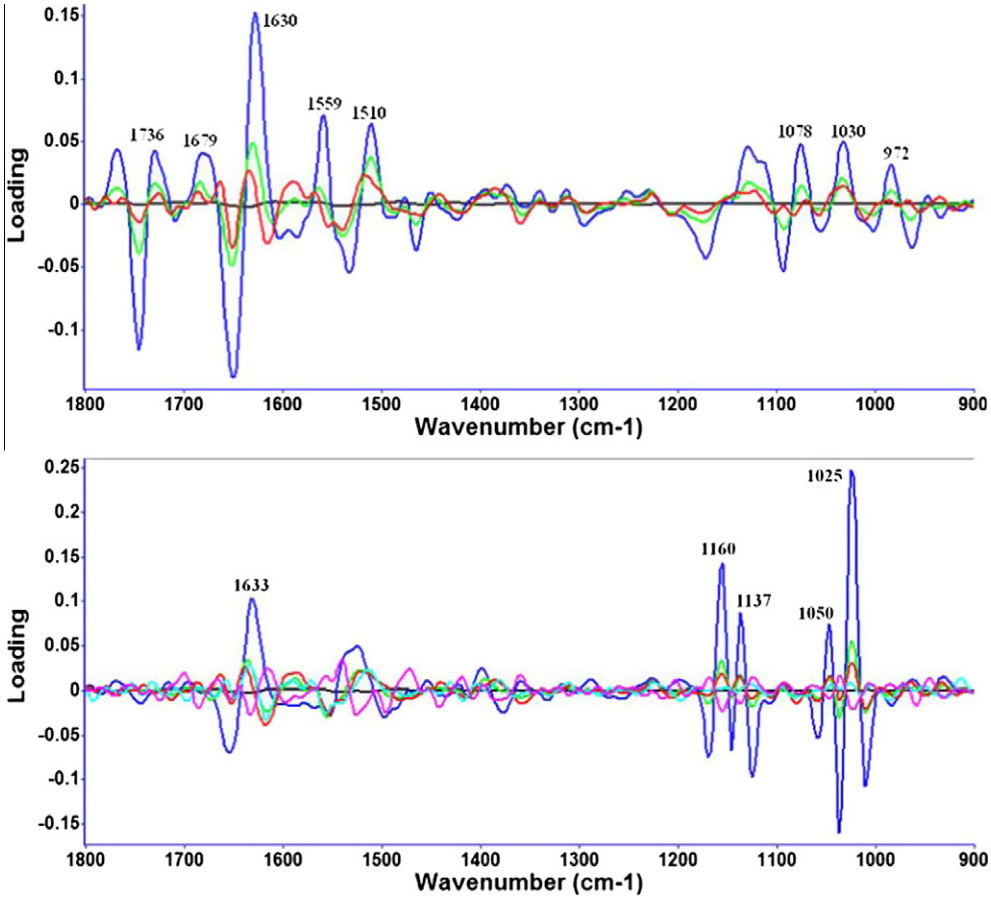

Fig. 3. Loading plots of the first three principal components obtained from (a) PC-DFA to explain segregation among onion and shallot and (b) PLSR to explain antioxidant capacity (PC1: blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

based on types. In the future, this rapid detection method may be used to determine TAC and TPC of other crops and vegetables.

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