Rapid prediction of phenolic compounds and antioxidant activity of Sudanese honey using Raman and Fourier transform infrared (FT-IR) spectroscopy

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Abstract

Fourier transform infrared with attenuated total reflectance (FTIR-ATR) and Raman spectroscopy combined with partial least square regression (PLSR) were applied for the prediction of phenolic compounds and antioxidant activity in honey. Standards of catechin, syringic, vanillic, and chlorogenic acids were used for the identification and quantification of the individual phenolic compounds in six honey varieties using HPLC–DAD. Total antioxidant activity (TAC) and ferrous chelating capacity were measured spectrophotometrically. For the establishment of PLSR model, Raman spectra with Savitzky-Golay smoothing in wavenumber region 1500–400 cm\(^{-1}\) was used while for FTIR–ATR the wavenumber regions of 1800–700 and 3000–2800 cm\(^{-1}\) with multiplicative scattering correction (MSC) and Savitzky–Golay smoothing were used. The determination coefficients (R\(^2\)) were ranged from 0.9272 to 0.9992 for Raman while from 0.9461 to 0.9988 for FTIR-ART. The FTIR–ATR and Raman demonstrated to be simple, rapid and nondestructive methods to quantify phenolic compounds and antioxidant activities in honey.

1. Introduction

Phenolic compounds are among the most chemically heterogeneous groups of compounds produced in plants. Phenolic acid and flavonoid compounds are the utmost important groups of phenolic compounds, with approximately 1000 compounds already reported (da Silva, Gauche, Gonzaga, Costa, & Fett, 2016). The concentration of these compounds depicts the quality of honey and accounts not only for antioxidant activity but also for its sensory features such as color (Lachman, Orsák, Hejtmánková, & Kovářová, 2010; Tahir, Xiaoobo, Zhihua, & Yaodi, 2015). Previous studies revealed the strong relationship between consumption of honey and low risk of cardiovascular diseases and cancer (Erejuwa, Sulaiman, & Ab Wahab, 2014; Tomasin, de Andrade, & Gomes-Marcondes, 2015).

Nowadays, a great attention has been directed to the use of natural antioxidants from honeybees and honey products. Antioxidant activity and phenolic compounds have become the most important factors to evaluate the quality and functional properties of honey (Lianda, Sant’Ana, Echevarria, & Castro, 2012). High-performance liquid chromatography combined with photo diode array detector or diode array detector (HPLC-PDA/DAD) is the conventional method used in the determination of phenolic compounds in honey. Despite its precision, however, conventional method is time-consuming, expensive and require a large amount of solvents (Kralj Cigic & Prosen, 2009). Therefore, simple, rapid and inexpensive technologies are required. Spectroscopic technologies are widespread in the analysis of main food components. They have also become popular in the analysis of honeys quality due to their benefits (including rapidity, directness, cost-effectiveness and usually no need for sample preparation). Near infrared (NIR) spectroscopy was successfully used to determine the total contents of phenolic, flavonoid and antioxidant capacity in honey as alternative to spectrophotometer (Escuredo, Carmen Seijo, Salvador, & Inmaculada González-Martin, 2013; Tahir, Zou, Shen, Shi, & Mariod, 2016). Fourier transform infrared (FT-IR) and Raman spectroscopy have feasible application in the assessment honey quality, which are used mainly to determine sugar, moisture and acidity (Anjos, Campos, Ruiz, & Antunes, 2015; Zhu et al., 2010; Özbalci, Boyaci, Topcu, Kadilar, & Tamer, 2013), and to discriminate honey botanical origin (Corvucci, Nobili, Melucci, & Grillenzi, 2015;
were kept for 10 min at 25 °C, the absorbance of the mixture was measured at 562 nm against a blank. The capacity of ferrous chelating was computed using the following formula:

$$\text{Chelatin capacity} \% = \left( \frac{A1 - A2}{A3} \right) \times 100$$

where A1 is the absorbance of the sample, A2 is the absorbance of the blank and A3 is the absorbance of the control. The control only contains FeCl and ferrozine (Kılıç & Yeşiloğlu, 2013).

2.3.3. Extraction of phenolic compounds

Extraction of phenolic compounds was conducted following the reported method (Gašić et al., 2014) with minor modifications. Honey samples (10 g) were dissolved in 40 mL of purified water, adjusted to pH 2 with HCl (0.1%) and sonicated for 15 min at 25 °C. Subsequently, the honey solutions were filtered through cotton wool to remove the solid particles. An SPE with C18 cartridges (Applied Separations Inc., Allentown, PA, USA) was conditioned by passing 10 mL of methanol and 10 mL purified water. The filtered honey solutions were loaded on the SEP cartridges and washed with 40 mL with acidified purified water (pH 2) to eliminate all sugars and other polar components of honey. The absorbed phenolic compounds were eluted with methanol (2 mL), and then the extracts were filtered through a 0.45-μm membrane filter prior to being quantified by HPLC-DAD.

2.3.4. HPLC-DAD analysis

A 1000 μg/mL standard solution of phenolic compounds was prepared in methanol. The working standard solutions were prepared by diluting the stock solution at 0.1, 2.1, 5, 50, 100, and 1000 μg/mL. All the standard solutions were stored in dark bottles at 4 °C. The Shimadzu LC 20A system (Tokyo, Japan) equipped with degasser (DGU-20A3), a binary pump (LC-20AD), autosampler (SIL-20 AC), communication bus module CBM 20A, and DAD detector SPD-M20A was used. The reversed-phase Zorbax SB-C18 column (250 mm × 4.6 mm, 5 μm particle size, Agilent Technologies) was used for separation of phenolic compounds. The mobile phase contained A (0.1% formic acid) and B (100% methanol). The gradient program was as follows: 0.0–5.0 min, 0–10% B; 5.0–20.0 min, 40% B; 20.0–32.0 min, 45% B; 32.0–45.0 min, 50% B; 45.0–70.0 min, 80% B; 70.0–75.0 min, 5% B. The injection volume for all samples was 5 μL. The injection volume for all samples was 5 μL. The flow rate was 0.2 mL/min. The results were monitored from 210 to 500 nm. The concentrations of phenolic compounds in the samples were quantified from the standard curves. The phenolic compounds in the samples were identified by comparing their retention times with authentic phenolic compound standards. Limits of detection (LOD) and limits of quantification (LOQ) were computed using standard deviations of the responses (SD) and the slopes of the calibration curves (S) based on the following formulas: LOD = 3 × (SD/S) and LOQ = 10 × (SD/S). The values of standard deviations and slopes were obtained from the calibration curves. Table S1 shows the retention time for different phenolic compounds in the samples sampled from various geographical and floral origins. The samples included the following: Acacia nilotica (n = 10), Acacia seyal (n = 20), Ziziphus spina-christi (n = 20), Ziziphus spina-christi (n = 20), Eucalyptus spp. (n = 10), Acacia seyal (n = 20), Ziziphus spina-christi (n = 20), and multi-floral (n = 30). The detailed information about the geographical and floral origins of samples was described in our previous work (Tahir et al., 2015).

2.2. Chemicals reagents

All solvents, phenolic compounds used for HPLC analysis were of HPLC grade and the rest of chemicals were of analytical grade. Catechin and phenolic acids (Chlorogenic, syringic, vanillic, p-hydroxybenzoic, p-coumaric, caffeic, ferulic, gallic and cinnamic acids) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were supplied by Sigma Chemical Co. USA. Ferrozine (2,4,6-Tris (2-pyrildyl)-s-triazine) was supplied by Sigma Chemical Co. Switzerland. Ferrous chloride, quercetin and methanol were supplied by Sinochem chemical reagent Co., Ltd China. Milli-Q water purification system (Millipore Corp., Billerica, MA, USA) was used for water purification.

2.3. Reference chemical analysis

2.3.1. Total antioxidant activity

The total antioxidant activity (TAC) was quantified using our previous method (Tahir et al., 2015, 2016). Measurements were conducted in triplicate and the mean value was stated as mg of quercetin equivalent antioxidant content (QEAC) per 100 g of honey.

2.3.2. Ferrous ion chelating activity

Ferrous ion chelating capacity was quantified following the assay reported recently (Xiong et al., 2013). In this experiment, 0.1 mL of ferrous chloride (2.0 mmol/L) was mixed 3.6 mL of honey dissolve in water 1:10 respectively. Thereafter, the reaction was started by 0.2 mL Ferrozine (5.0 mmol/L). The reaction mixtures were kept for 10 min at 25 °C, the absorbance of the mixture was measured at 562 nm against a blank. The capacity of ferrous chelating was computed using the following formula:

$$\text{Chelatin capacity} \% = \left( \frac{A1 - A2}{A3} \right) \times 100$$

where A1 is the absorbance of the sample, A2 is the absorbance of the blank and A3 is the absorbance of the control. The control only contains FeCl and ferrozine (Kılıç & Yeşiloğlu, 2013).

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2.4.1. FTIR-ART spectroscopy

For measurement of honey spectra, approximately 300 µL of each honey was investigated on a Thermo Scientific Nicolet iS50 FT-IR Spectrometer (Thermo Fisher, USA) using a diamond single reflection attenuated total reflectance (ATR) accessory equipped by a zinc selenide crystal. The software OMNIC version 8 was used for spectral acquisition. Infrared spectra were obtained in the range of 4000–600 cm\(^{-1}\) with a spectral resolution of 4 cm\(^{-1}\). The averaged spectra were obtained using 32 scans including subtraction of a background scan of the clean diamond crystal. The diamond was cleaned between samples using alcohol.

2.5. Raman spectroscopy

Spectra of the honey samples were acquired on a DXR Laser Raman Spectrometer (Thermo Fisher, USA) coupled with excitation

![Figure 1](image-url)
laser: 532 nm; the spectrometer allows a resolution of 5 cm\(^{-1}\) using 900 lines/mm. Each honey was investigated, using a laser power at the sample of 10 mW. Approximately 500 L of each honey was positioned on a glass slide; the visual center was correctly identified through the microscope, and then the spectrum was collected. A spectrum from each honey sample was collected for 5 min, using the continuous extended scan from 50 to 3500 cm\(^{-1}\).

The full spectra of honey samples indicates the presence of some regions that incorporate noises to the calibration models caused by interferences mainly water. Thus, only the fingerprints regions were investigated: 1500–400 cm\(^{-1}\) for Raman while for FT-IR were 1800–700 and 3000–2800 cm\(^{-1}\). The number of variables for Raman and FT-IR was 1142 and 2699, respectively.

### Table 1

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<td>11.38</td>
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</table>

Sample number, n; quercetin equivalent antioxidant content (QEAC); Standard deviation, SD; Maximum, Max; Minimum, Min; Coefficient of variation, CV; CV = \([(SD/\text{Mean}) \times 100]\).

### 3. Results and discussions

#### 3.1. Phenolic compounds and antioxidant activity in honey

Several phenolic compounds have been monitored in Sudanese honey samples (Table S2). Vanillic acid, chlorogenic acid and syringic acid and catechin were detected in all honey samples analyzed, while Gallic acid was not detected in any samples. Therefore, the predominant phenolic compounds were selected for developing prediction models. The reference chemical data are stated as the mean, standard deviation, and coefficient of variation. The descriptive statistics depicted in Table 1, showed a great variation in phenolic compounds and antioxidant activities in the calibration and external validation sets. This variation was due to the differences of the geographical and floral sources of honey samples (Tahir et al., 2016). From Table 1 it can be concluded that the Sudanese honey samples contain a considerable amount of bioactive compounds with varied concentrations, revealing a significant antioxidant capacity. The range of six different measurements in the calibration set covered the range in the prediction set and it is clear that their standard deviations were not significantly different. Thus, the distribution of the chemical data in the calibration and prediction sets was appropriate for developing models to determine these parameters in honey.

#### 3.2. Spectra interpretations

FTIR and Raman spectra of different kinds of honey analyzed are shown in Fig. 1. The spectra procured in this work are comparable with those described in the literatures (Anjos et al., 2015; Pierna et al., 2011; Özbalci et al., 2013). Regarding the TFFIR spectra, as could be seen in Fig. a and b, the peaks in the region...
Calibration statistics for phenolic compounds content and antioxidant activities determined using PLSR for Raman and FT-IR Spectroscopy. The bands at 1700–1600 cm\(^{-1}\) were originated to stretching band of carbonyl groups C=O and C=C stretching, this region was found to be related to phenolic molecules (Preserova et al., 2015). According to Gok et al. (2015), the vibration in the region 1540–1715 cm\(^{-1}\) is the result of deformations of O–H, C=O–H and C=O=C. As well, the same spectral region corresponds to flavanol and phenol (Masek et al., 2014). The vibrations in the regions 940–1175 cm\(^{-1}\) are due to C–OH group as well as the stretches C–C and C–O in the carbohydrate structure and C–O in the phenol (Masek et al., 2014).

In Fig. 1c and d, the Raman spectra were characterized by the intense bands around 2941 and 2903 cm\(^{-1}\) was assigned to C–H stretching, and the vibrational observed in the low wavenumber regions 200–500 cm\(^{-1}\) has been assigned to C–C–C, C–C–O, C=O and C–C=O (Ozbalci et al., 2013). The band at 518 cm\(^{-1}\) was originated from strong deformation of C–C–C and C–C–O. The strong band at 630 cm\(^{-1}\) was assigned to ring deformation. The bands around 705 cm\(^{-1}\) originated to stretching C–O, C–C–O and O–C–O bending and the bands in region 822–866 cm\(^{-1}\) were assigned to the C–H, O–H–H, and CH\(_2\) bending. The vibration around 95 cm\(^{-1}\) was related to C–H and C–O–H (Zhu et al., 2010). The peak at 1075 cm\(^{-1}\) was assigned mainly to bending vibration of C–H and C–O–H in carbohydrates and slight contribution by the vibration of C–N bond in proteins and amino acid. The peak at 1127 cm\(^{-1}\) could be due to the combination of stretching vibration of C–O bond and vibration of C–N bond of carbohydrate and amino acids, respectively. A strong peak at 1266 cm\(^{-1}\) was found to be due to the deformation vibration of C–C–H, O–C–H, and C–O–H. The absorbance band at 1460 cm\(^{-1}\) was originated from the combination of bending vibration of CH\(_2\) and vibration of COO– group. Besides, the same spectral region has been attributed to the flavanol and organic acids (Nickless et al., 2014; Paradkar & Irudayaraj, 2002). The small band observed at 1365 cm\(^{-1}\) might be attributed to the symmetric deformation in the plane of CH\(_2\) (Pierna et al., 2011).

### 3.3. Quantitative analysis

Table 2 displays the results of PLSR analysis using both FT-IR and Raman data for quantitative determinations of catechin, chlorogenic, syringic, vanillic acids, TAC and ferrous chelating capacity in honey.

### 3.3.1. Results of PLSR models

PLS is of special interest because, it can analyze data with strongly collinear correlated, noisy, and various X-variables, and also simultaneously model several response variables, Y, i.e., profiles of performance (Wold et al., 2001). PLS and large X-variables showed good quantitative results in many studies (Durakli et al., 2017; Friedel et al., 2013; Georgouli et al., 2017; Tahir et al., 2016; Wu et al., 2016; Ozbalci et al., 2013). PLSR models were constructed using the spectra set both in full cross-validation and prediction set. PLSR models were performed using selected regions for FT-IR and Raman. The calibration models were developed using Raman and FT-IR spectra set of the randomly chosen 64 honey samples as shown in Table 2. The robust PLSR model must have a high correlation coefficient, low RMSECV, RMSEP and a small number of LVs, preferably less than ten (Abdi, 2010). Good calibration models were achieved both in FT-IR and Raman regions. In fact, R\(^2\)c values in cross-validation were higher than 0.95 and RMSECV values for all parameters analyzed were relatively low and ranged from 0.40 to 1.14 and 0.33–0.86 for Raman and FT-IR models, respectively. The performance of the models of phenolic compounds and antioxidant activities in honey was confirmed using the remaining 36 honey samples. Both techniques revealed excellent capability in predicting the concentrations of phenolic compounds and antioxidant activities in different honey samples, as shown high R\(^2\)p values (>0.92). Although the RMSEP of TAC in both FT-IR and Raman seem to be slightly higher but still remains good.

These findings revealed that the PLSR models successfully captured essential FT-IR and Raman spectra features for phenolic compounds existing in different honey samples. Figs. 2 and 3 represent the regression lines of PLSR models for catechin, chlorogenic, syringic, vanillic acids, TAC and ferrous chelating activity

<table>
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N: number of samples; LVs: latent variables; SD: standard deviation; RMSECV: Standard Error of Cross-Validation; RMSEP: root mean square error of prediction; R\(^2\): multiple correlation coefficients in the calibration set (R\(^2\)c) and prediction set (R\(^2\)p); QEAC: quercetin equivalent antioxidant content.
obtained by FT-IR and Raman, respectively. All constructed calibration models in the FT-IR and Raman regions presented excellent results for the six parameters measured. Although the results of ferrous chelating capacity in FT-IR and Raman were moderately good, the predicted results of TAC were not good as compared to those obtained for the other parameters. We expected these results for antioxidant activities since the FT-IR and Raman spectra represent functional group characteristics of specific chemical constituents in honey; but, FT-IR and Raman do not reflect possible interactions and synergistic effects (competitive inhibitions) amid honey antioxidants. These features, perhaps are related to the free radical reaction mechanisms and could describe why the results of the most phenolic compounds in honey exhibited greater $R^2_p$ values compared to those $R^2_p$ of antioxidant activity.

Finally, the findings revealed that the FTIR and Raman spectroscopy could be used as a simple and direct method to enable the apiculture and pharmaceutical industries to efficiently assess the antioxidant activity and health benefits compounds.
Data fusion process seeks to integrate data from the same set of samples collected from multiple blocks into a single model which can lead to enhancement of the accuracy of the predictions more than single block achieved. Although both Raman and FT-IR showed good results, however, the results of vanillic acid and TAC were not good enough compared to other parameters measured. Thus, data fusion method has been applied to investigate whether the combination of spectra can improve the prediction capacity of these parameters. In this work, PLSR model based on data fusion was developed. For integrating FT-IR and Raman, the spectra were scaled using simple mean-centering and variance scaling prior to combining spectra to give alike weights to the data obtained from techniques of different features. Afterward, the spectra of two techniques fused in a single matrix had the number of columns equal to the number of honey samples and the number of rows equal to the number of a total number of data (selected regions) from FT-IR and Raman. Fig. 4 represents the correlation plots of chemical data against predictions for the six parameters obtained from combined spectra. As depicted in Table 2, the

3.3.2. Results of PLSR models based on data fusion

Fig. 3. Correlation plots for the prediction of catechin, chlorogenic acid, syringic acid, ferrous chelating activity and quercetin equivalent antioxidant content (QEAC) using PLS based on the Raman spectra.
determinations performed using the Raman regions gave slightly better results than those using the FT-IR regions, which was in agreement with results reported in the literature (Wu et al., 2016). This might be attributed to the differences of the vibrational features of functional groups in the two technologies. Comparison of the Raman results with the fused spectra showed that the R² values were nearly similar, demonstrating that the added FT-IR data is relatively not used. Among the six parameters analyzed the fusion of the selected regions slightly improved the prediction of syringic and vanillic acids. Several authors reported that the data fusion did not always enhance individual results (Apetrei et al., 2012; Cosio, Ballabio, Benedetti, & Gigliotti, 2007; Masnan et al., 2012) and in some cases was influenced negatively (Roussel, Bellon-Maurel, Roger, & Grenier, 2003). These findings revealed that the data fusion of FT-IR and Raman does not significantly enhance prediction results and in some parameters had negative effect (Table 2).

4. Conclusion

This work represents the first study to examine the capability of FTIR and Raman spectroscopy combined with chemometrics for...
the prediction of TAC, ferrous chelating capacity catechin, syringic, vanillic, and chlorogenic acids in honey. For constructing PLSR models, the vaneweenrange 1500–400 cm\(^{-1}\) was selected for Raman, while for FT-IR, 1800–700 and 3000–2800 cm\(^{-1}\) were utilized. It was noted that the performances of models based on the Raman spectra were slightly better than those based on the FTIR spectra. The prediction results of vanillic acid and TAC for both techniques were not good compared to those of other parameters measured. Therefore, the combination of the two spectra was used to examine whether it can improve the prediction results. We noted that a combination of two complementary data for the same sample, i.e. FT-IR and Raman spectroscopy did not improve prediction models. Regardless of the results of combined data, both Raman and FT-IR showed satisfactory prediction capacity for all parameters investigated. The results demonstrated that the developed PLS models based on Raman and FTIR were superior to those established using NIR spectra (Escoiredo et al., 2013; Tahir et al., 2016). Since this work is a feasibility study, further study with a large number of honey varieties from different countries are required prior such an approach is accepted by the apiculture and pharmaceutical industries with confidence.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.01.024.

References


Tomasin, R., de Andrade, R. S., & Gomes-Marcondes, M. C. C. (2015). Oral Administration of Aloe vera (L) Burm. f. (Xanthorrhoeaceae) and Honey improves the host body composition and modulates protein synthesis through
reduction of tumor progression and oxidative stress in rats. *Journal of Medicinal Food.*


