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The famous Turkish rose essential oil: Characterization and authenticity monitoring by FTIR, Raman and GC–MS techniques combined with chemometrics

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ABSTRACT

There is a necessity for rapid, robust, easy, accurate and cost-effective methodologies for the quality control of essential oils from medicinal and aromatic plants. *Rosa damascena* essential oil is a high-value natural product with its unique quality properties and economic importance. This research evaluated the capability of Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy and gas chromatography-mass spectrometry (GC–MS) techniques combined with chemometrics for determination of the authenticity of *R. damascena* essential oil. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were successfully employed with 100% accuracy for discrimination of authentic *R. damascena* essential oil samples from fraudulent commercial samples. Consistent results were obtained by FTIR, Raman and GC–MS techniques. Two of twenty commercial samples were determined as authentic *R. damascena* essential oil samples using the three analytical techniques. Findings showed that FTIR and Raman spectroscopy combined with chemometrics could be used as reliable, robust, rapid, accurate and low-cost analytical techniques for quality evaluation of *R. damascena* essential oil.

1. Introduction

Rosa damascena belongs to the genus Rosa and is generally known as Damask rose or oil-bearing rose. According to the previous reports, genus Rosa contains at least 200 species, but *R. damascena* (the most aromatic species) has special importance among Rosa species by virtue of its unique quality properties (Nedeltcheva-Antonova, Stoicheva, & Antonov, 2017). *R. damascena,* also known as pink rose oil, has an intense and pungent scent when compared to other rose species.

Essential oil, the high-value natural product obtained from the *R. damascena,* has high economic importance with its broad application areas such as perfumery, pharmaceuticals, cosmetics and food industries (Baydar, 2015). Most *R. damascena* is produced in Bulgaria and Turkey. Turkey is the leading producer of *R. damascena,* and almost all of the essential oil is exported globally; in this way, 50% of the demand for *R. damascena* essential oil is supplied by Turkey.

Turkish Rose Oil is known as the rose oil, obtained from the *R. damascena* roses, which are cultivated largely in Isparta, Burdur, Afyon and Denizli in Turkey (Ormeci & Demircan, 2012). Cultivation of *R. damascena* roses began in Istanbul in the 1880s through the encouragement of Ottoman Sultan Abdulhamid II (1842–1918). *R. damascena* roses were grown in many regions of Turkey in those years. However, cultivation was restricted to Isparta and Burdur provinces because of the soil and climatic requirements of the *R. damascena* (Yaniv & Dudai, 2015). Today, the oil rose *R. damascena* is an emblematic rose species for both Isparta and Turkey.

Monitoring of the authenticity of *R. damascena* essential oil has great importance for both the protection of the consumer and quality properties. Authentic *R. damascena* essential oil does not contain any additional substances or any impurities. The quality parameters of *R. damascena* are determined by international standards. However, there is a need for fast, economical, reliable, selective, non-destructive and robust methodologies that have the capability to detect any counterfeit or adulterated essential oil product. It would be possible to protect the quality of authentic *R. damascena* essential oil by application of the new methodologies which detect frauds or any counterfeit essential oil product.

Few published studies have attempted to determine the authenticity of *R. damascena* essential oil. In these studies, electronic nose and enantioselective gas chromatography was used to determine the

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authenticity of *R. damascena* essential oils (Gorji-Chakespari et al., 2017; Krupčík et al., 2015).

Vibrational spectroscopy is known as a reliable and robust analytic technique since it provides fingerprint information about the composition and molecular structure of any material (Sivakesava & Irudayaraj, 2001). Recent studies have shown that vibrational spectroscopy combined with chemometrics was successfully applied for the determination of the authenticity of various essential oils. These previous studies mainly focused on two issues: the first was the determination of the authenticity of essential oils; the second was the detection of adulteration in essential oils. For example, Bombarda et al. (2008) developed a fast test methodology for the determination of the geographical origin and authenticity of lavender essential oil using Fourier transform infrared (FTIR) spectroscopy and chemometrics. In a different piece of research, FTIR and Raman spectroscopy were implemented for quality control of eucalyptus essential oil from Australia and China (Baranska et al., 2006). Results from earlier studies demonstrate the strong capability of vibrational spectroscopy combined with chemometrics for assessment of the authenticity and adulteration in essential oils.

The present research study evaluates the effectiveness of FTIR, Raman and gas chromatography-mass spectrometry (GC–MS) techniques combined with chemometrics for discrimination and classification of *R. damascena* essential oil. Up to now, no study has evaluated the authenticity of *R. damascena* using vibrational spectroscopy combined with chemometrics. Application of FTIR and Raman techniques, in combination with chemometrics, is the main innovation of the present research study. Additionally, the GC–MS technique, combined with supervised chemometrics, was applied for the same aim based on the volatile composition of *R. damascena* essential oils.

2. Materials and methods

2.1. Apparatus

Raman spectral acquisition was performed using a Raman Rigaku Progeny X2 spectrometer (Rigaku Raman Technologies, Wilmington, DE, USA) in the spectral range of 200 and 2000 cm^{-1}. Xantus V3.0.0.0 software was used for spectral acquisition and instrument control. A Bruker Tensor 27 spectrometer (Bremen, Germany) was used for the measurement of FTIR spectra. A single-bounce ATR accessory, with a diamond crystal, was used for all FTIR measurements. The GCMS-QP2010 gas chromatography-mass spectrometer system (Shimadzu, Japan) was used to obtain total ion chromatograms of samples. Volatile compounds were determined by using commercial mass spectra libraries (NIST27 and WILEY7).

2.2. Materials

The authentic *R. damascena* essential oil samples (n = 12) were obtained from credible essential oil producer companies (Isparta, Turkey). Commercial *R. damascena* essential oils (n = 20) were bought from different brands (Turkey). Diethyl ether (Merck-Schuchardt, FRG, GC > 98%) was used for sample preparation of GCMS analysis.

2.3. Raman analyses

All samples were kept at room temperature (25 °C) for 30 min; then, they were placed in measurement vials of the Raman spectrometer. Raman measurements were performed directly through Raman vials without any sample preparation. The spectral acquisition was performed by using a 1064 nm YAG laser at the spectral range of 2000–200 cm^{-1}. Laser power and exposure time were selected as 0.25 W and 0.851 s, respectively. Four measurements were gathered for each sample, and an average spectrum was obtained prior to the chemometrics.

2.4. FTIR analyses

All samples were kept at room temperature (25 °C) for 30 min prior to the FTIR analyses. An ATR accessory (single-bounce) was used in all spectral acquisition. Spectral measurement parameters of resolution and accumulation were selected as 4 cm^{-1} and 16 scans, respectively. OPUS program Version 7.2 (Bruker Gmbh) was employed for instrument control and data acquisition. Each sample was placed on a diamond ATR crystal with the help of a pasteur pipette. The ATR crystal was cleaned with ethanol (80% v/v) prior to each spectral acquisition. The background air spectrum was scanned before each acquisition.

2.5. GC–MS analyses

GC–MS analyses of all samples (n = 32) were performed with some modifications to the method of Zhu et al. (2017). GC–MS analyses were performed by using a Restec (Bellefonte, USA) Rtx-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm). A gradient oven temperature was selected. The initial oven temperature was kept at 40 °C for 3 min; then, the temperature was gradually increased to 100 °C at the rate of 8 °C/min. It was then raised to 200 °C at the rate of 5 °C/min, and finally to 250 °C at the rate of 10 °C/min. It was kept constant for 10 min at the temperature of 250 °C. The temperature of the injection block and the detector was adjusted to 250 °C. The carrier gas was helium at 1 mL/min flow rate. All samples were diluted at 1:100 in diethyl ether. Three μL of diluted samples were injected into GC–MS with a split ratio of 1/50. The mass scan was set for acquisition from 35 to 650 (*m/z*). The identification of volatile compounds was performed by comparing the total ion chromatograms with the commercial mass spectra libraries (NIST27 and WILEY7). Quantification of each volatile compound was performed on the basis of the relative area of the total ion chromatogram (TIC) peaks of volatile compounds.

2.6. Chemometrics

Chemometrics of hierarchical cluster analysis (HCA) was applied to the FTIR and Raman data of *R. damascena* essential oils and commercial samples using the OPUS software version 7.2 (Bruker, Germany). Dissimilarities and similarities were clearly observed by HCA dendrogram. The HCA dendrogram was built using second derivative spectra (9 smoothing points), Euclidian distance and Ward's algorithm.

Principal component analysis (PCA) was performed using JMP 9 software (SAS, Cary, NC, USA). Two dimensional PCA plots were obtained by using for displaying the scattering pattern of authentic and commercial samples. The spectral range of 1800–500 cm^{-1} and 2000–200 cm^{-1} was used in the FTIR and Raman analyses, respectively.

SIMCA 15 (Umetrics, Umea, Sweden), as a supervised chemometrics technique, was used to perform HCA and PCA analyses of GC–MS data, which were obtained from *R. damascena* essential oil samples (n = 12) and commercial (n = 20) samples.

3. Results

3.1. Analysis of FTIR spectra of R. damascena essential oil

The typical FTIR spectrum of *R. damascena* essential oil and overlaid FTIR spectra of *R. damascena* essential oil samples and commercial samples are presented in Fig. 1(A) and (B), respectively. The FTIR spectrum of *R. damascena* essential oil had significant vibrational bands at 3345, 2960, 2922, 2853, 1668, 1515, 1451, 1377, 1260, 1235, 1053, 1004 and 829 cm^{-1}. The spectral band at 3345 cm^{-1} could be assigned to the stretching vibrations of the OH functional group of alcohols (Sandasi et., 2011; Tankeu., 2014). The band with a peak point at 2960 cm^{-1} may be attributed to the C═C–C ring vibrations of volatile compounds (Tankeu et al., 2014). Two bands at 2922 and 2853 cm^{-1} were assigned to the methylene C—H asymmetric and symmetric stretching

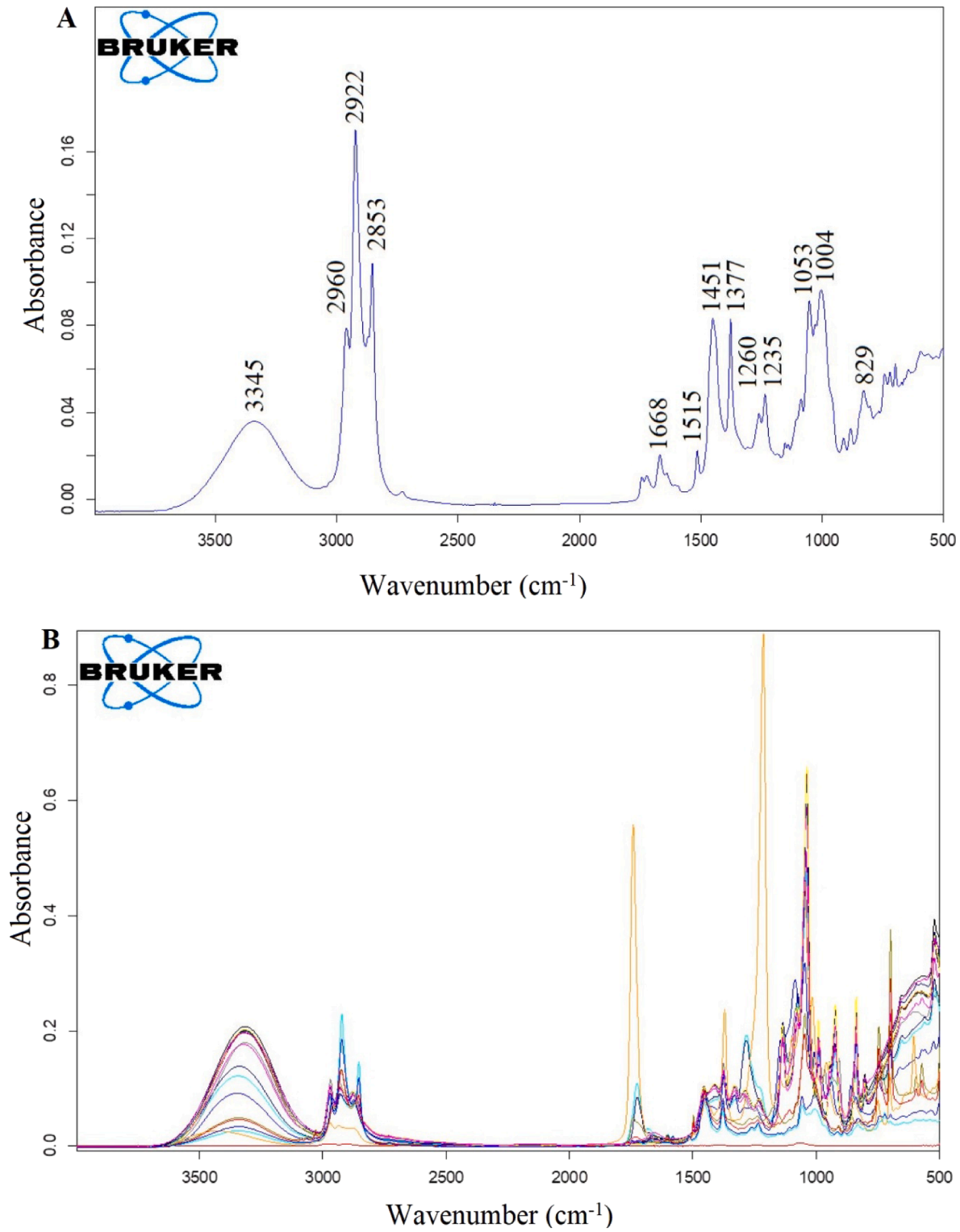


Fig. 1. (A) The typical FTIR spectrum of *Rosa damascena* essential oil. (B) Overlaid FTIR spectra of *Rosa damascena* essential oil samples and commercial samples.

vibrations, respectively (Berechet et al., 2015). The band with a peak point at 1666 cm^{-1} corresponded to the C═C stretching vibrations (Yang et al., 2005). The peak around 1515 cm^{-1} originated from the aromatic ring C═C skeleton vibrations of aromatic substances (Li et al., 2013). The strong bands at 1451 and 1377 cm^{-1} were assigned to the C-OH bending vibrations and C—H asymmetric + symmetric bending vibrations, respectively (Berechet et al., 2015; Li et al., 2013). Bands at 1260 and 1235 cm^{-1} resulted from the C—C—O stretching vibrations and C—O stretching vibrations of phenolics (Bardakci & Secilmis, 2006). The significant bands at 1053 and 1004 cm^{-1} could be attributed to the hydroxyl group vibrations and methylene vibrations, respectively (Berechet et al., 2015; Sandasi et al., 2011). The band at 829 cm^{-1} could be assigned to the C—H stretching vibrations (Tavares & Noreña, 2020). Additionally, the overlapped spectra of all samples are presented in Fig. 1(B). As can been seen, significant spectral diversities were observed between samples.

3.2. Determination of the authenticity of R. damascena essential oil based on FTIR data

R. damascena essential oils and commercial samples were classified based on their FTIR spectra using HCA and PCA. HCA was used to observe the relationship between authentic samples and commercial samples.

What is striking about the HCA is it presents a dendrogram, in which all samples clustered related to their similarities and differences. In other words, HCA groups the elements into clusters in terms of their nearness in the multidimensional space (Li et al., 2012). In the present study, HCA was implemented in the spectral range of 1800–500 cm^{-1} by using 2nd derivative FTIR spectra through Ward's algorithm. The superiority of Ward's algorithm is that it provides well-separated clusters by minimizing the variance between groups (Taylan et al., 2020). Calculations were performed using the Euclidian distance and dendrograms were built by Ward's algorithm (OPUS Version 7.2, Bruker, Germany). Fig. 2(A) presents the HCA dendrogram, revealing the similarity and disparity patterns of all samples with a high heterogeneity value of 10000. All samples were clustered into two main groups: authentic *R. damascena* essential oil samples were clustered on the left arm (numbered as 1) of the dendrogram, and fraudulent samples were clustered on the right arm of the dendrogram (numbered as 2). In this way, authentic *R. damascena* essential oil samples were clearly distinguished from fraudulent or non-authentic samples. Results state that only two of the twenty commercial samples were clustered between authentic samples. In other words, one can conclude that two of the commercial samples (T1, T5) were *R. damascena* essential oil and rest of the commercial samples included impurities or foreign compounds in their compositions. Favorably, the HCA dendrogram provided visual (2D-graph) presentation of clusterization characteristics of samples with details about the relationship between clusters and sub-clusters.

Previous research has reported that PCA is used to find the main variability sources in a data set and the relationship between/within objects and variables (Leopold et al., 2011). Further, PCA is known as a pattern recognition technique which is used for classification and

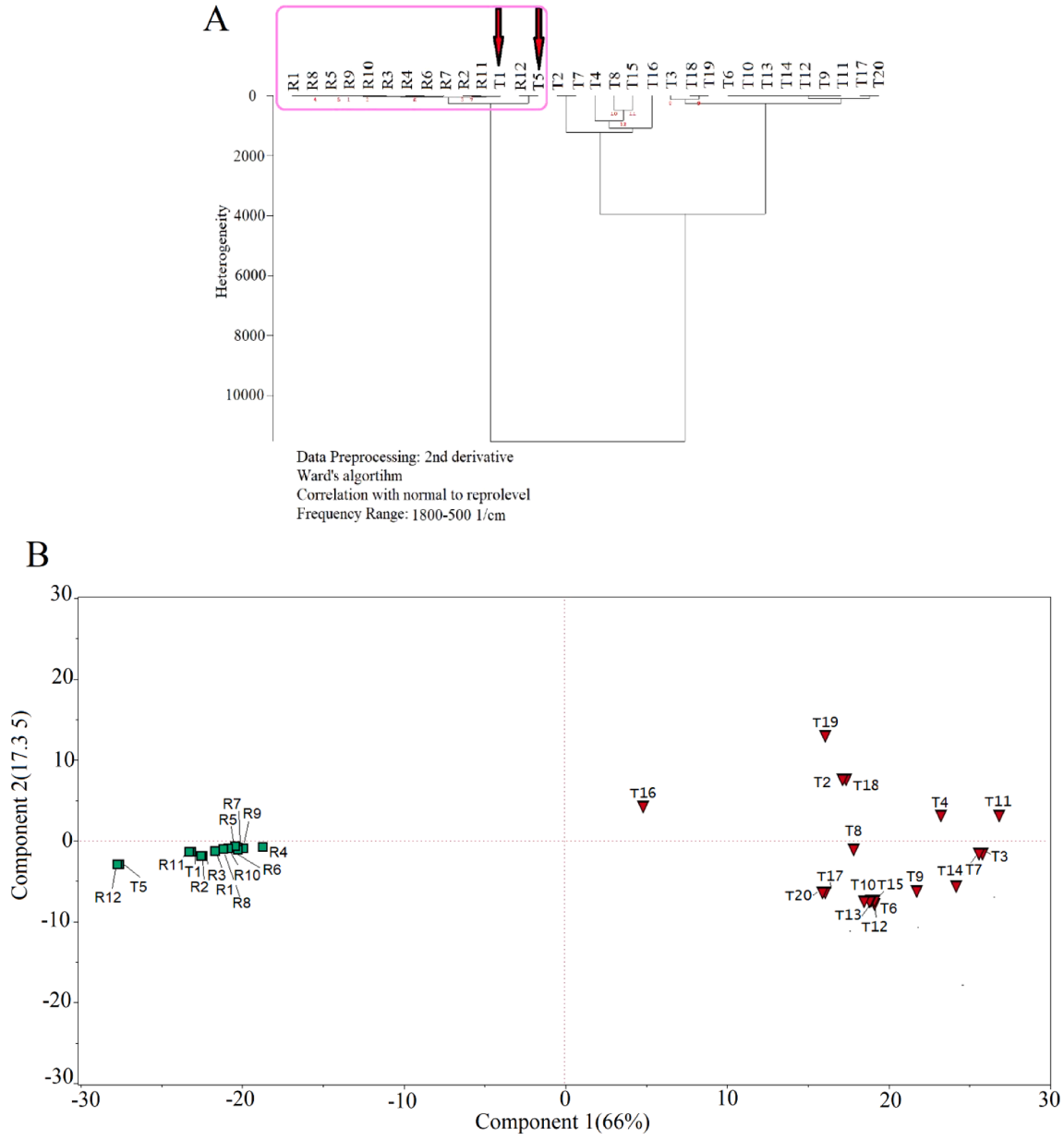


Fig. 2. (A) HCA dendrogram (Ward's algorithm) of FT-IR spectra from *Rosa damascena* essential oil samples (n = 12) and commercial samples (n = 20). (B) Two dimensional PCA score plot for *Rosa damascena* essential oil samples (n = 12) and commercial samples (n = 20).

discrimination of samples on the basis of experimental scientific findings (Rahmania et al., 2015).

The PCA analyses were performed to observe classification patterns of authentic and commercial samples on the basis of their FTIR and Raman spectra. Fig. 2(B) shows the PCA results (PC1 vs PC2) from the FTIR spectra for the spectral range of 1800–500 cm^{-1}. Selection of the wavenumbers between 1800 and 500 cm^{-1} provided clear classification and discrimination of investigated samples according to their authenticity. As it can be seen from Fig. 2(B), clear discrimination was observed between authentic *Rosa damascena* samples and fraudulent commercial samples. Two well separated groups were observed. Authentic samples (R1-R12, T1, T5) were marked with green squares (left side of the graph) and fraudulent samples were marked with red triangles (right side of the graph). Only two of the commercial samples (T1 and T5) were located between authentic samples in the 2-D PCA plot. Five principle components (PCs) represent the 98.37% of total variance. The first two PCs explained 83.3% of the total variance among samples. PC1, PC2, PC3, PC4 and PC5 described the 66.04%, 17.33%, 7.49%, 4.33% and 3.18% of total variance, respectively. Eigenvector plots of the PCA are presented in the supplementary file (S1). The discrimination of all of the samples could be attributed to the compositional differences between samples, which are revealed by the fingerprinting FTIR spectroscopy. Obtained results were 100% compatible with those obtained by the HCA.

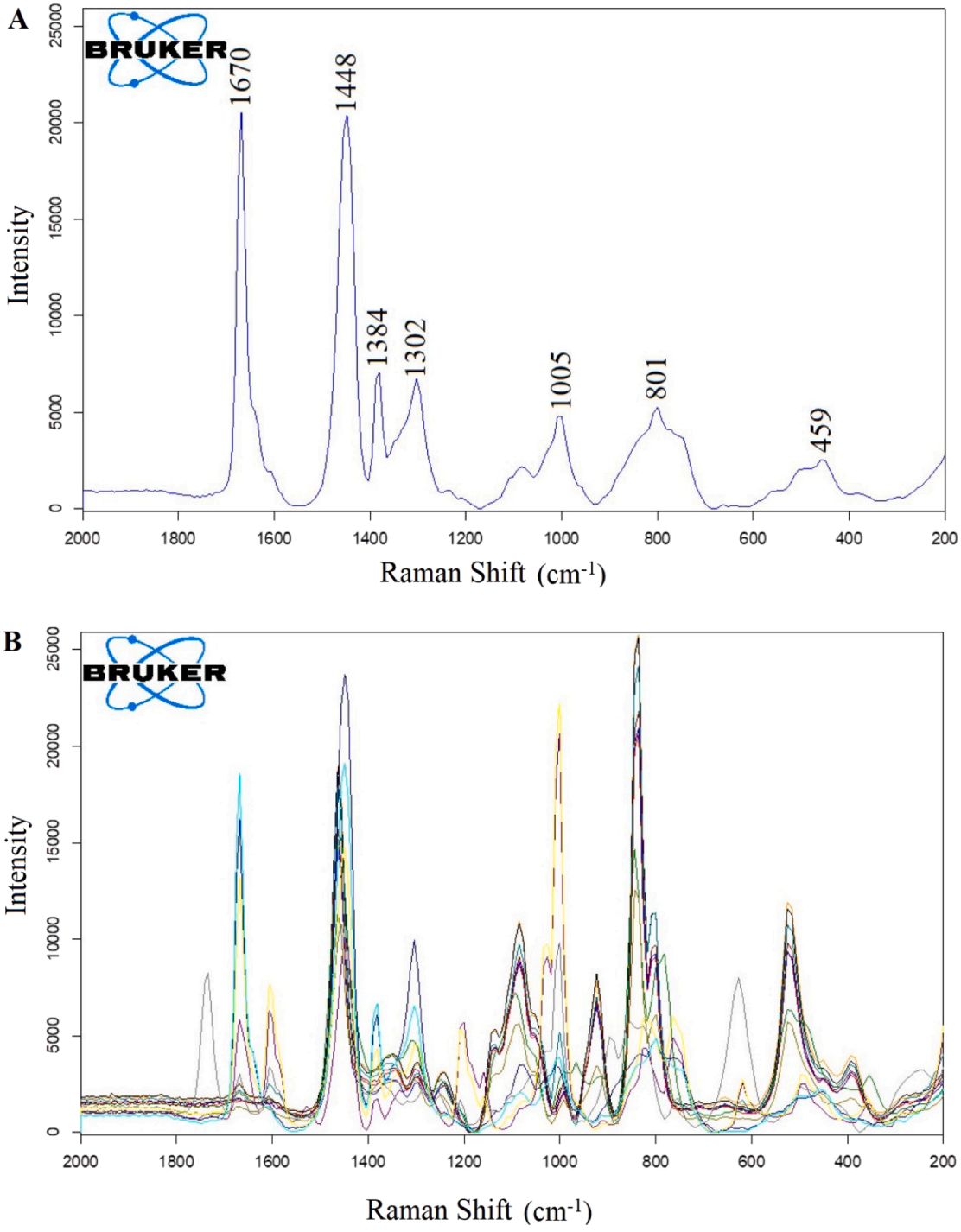


Fig. 3. (A) The typical Raman spectrum of *Rosa damascena* essential oil. (B) Overlaid FTIR spectra of *Rosa damascena* essential oil samples and commercial samples.

3.3. Analysis of Raman spectra of R. damascena essential oil

The characteristic Raman spectrum of *R. damascena* essential oil and overlaid Raman spectra of *R. damascena* essential oil samples and commercial samples are presented in Fig. 3(A) and (B), respectively. Main Raman vibrational bands were observed at 1670, 1448, 1384, 1302, 1005, 801, and 459 cm^{-1}. The band with a peak point at 1670 cm^{-1} was attributed to the C═C stretching vibrations (Baranska et al., 2006; Jentzsch et al., 2015). The spectral band at 1448 cm^{-1} was assigned to the CH_3/CH_2 bending vibrations (Jentzsch et al., 2015; Sandasi et al., 2011). The band at 1384 cm^{-1} may be related to the CH_3 bending (attached to a C═C) vibrations and = CH rocking vibrations, respectively (Jentzsch et al., 2015). The vibrational band at 1032 cm^{-1} is due to the C—H twisting vibration of the $–CH_2$ group (Nedeljkovic et al., 2017). Three wide bands at 1005, 801 cm^{-1} were attributed to the CH bending vibrations, C—O stretching (alcohol) vibrations, respectively (Jentzsch et al., 2015; Wang et al., 2014).

3.4. Determination of the authenticity of R. Damascena essential oil based on Raman data

The HCA dendrogram revealed the intrinsic relationship between authentic *R. damascena* essential oil samples and commercial samples. In other words, the location of a sample on the HCA dendrogram presents the similar characteristics of that sample. The HCA collects the most similar spectra into the same cluster. In the present study, Euclidian distance was used though Ward's algorithm for building the HCA clusters in the spectral range of 2000–200 cm^{-1} by using 2nd derivative

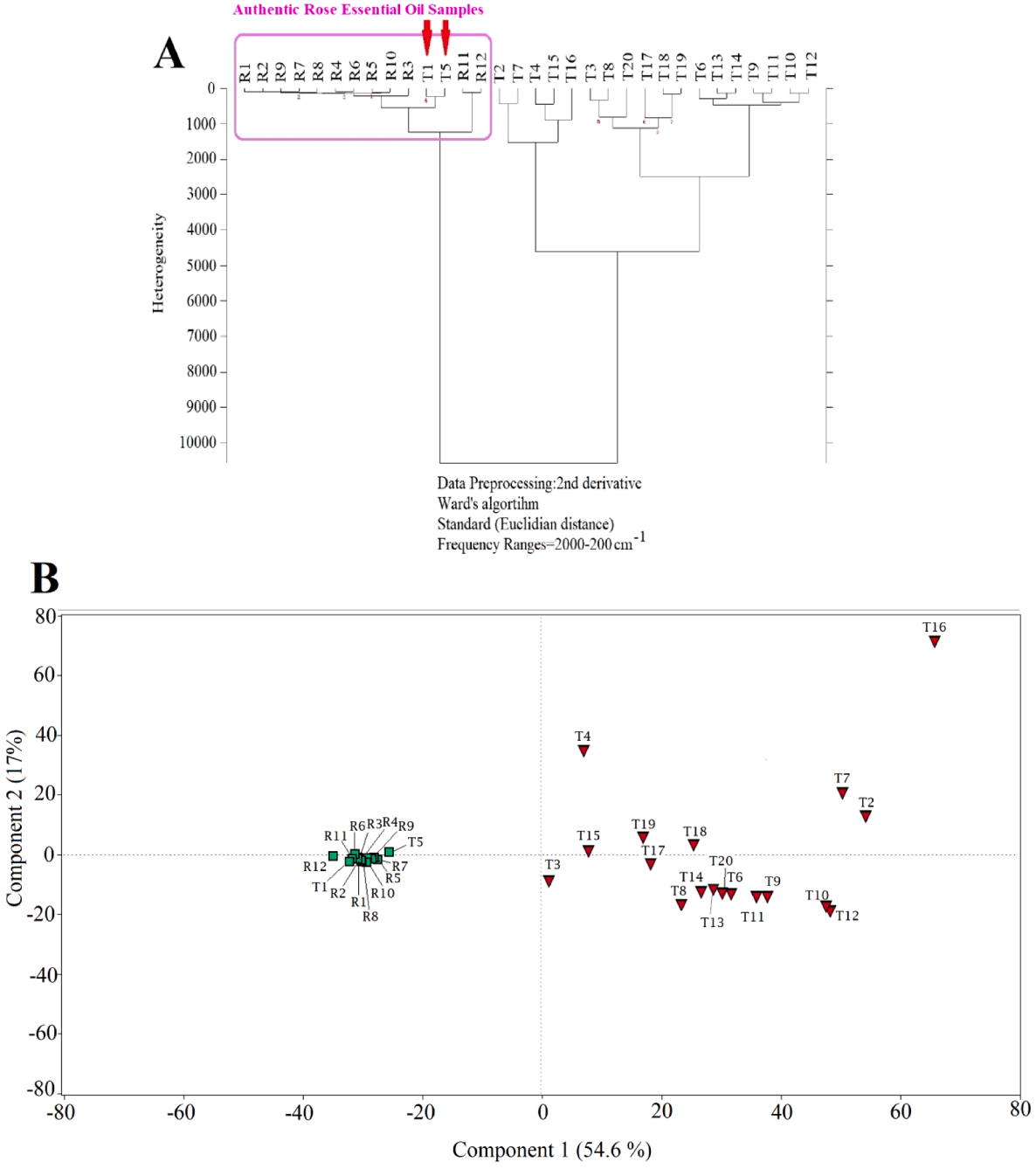


Fig. 4. (A) HCA dendrogram (Ward's algorithm) of Raman spectra from *Rosa damascena* essential oil samples (n = 12) and commercial samples (n = 20). (B) Two dimensional PCA score plot for *Rosa damascena* essential oil samples (n = 12) and commercial samples (n = 20).

Raman spectra (OPUS Version 7.2, Bruker, Germany). Ward's algorithm minimized the loss of information related to each group. Tree-like, two-dimensional dendrograms present the closely-related and distantly-related samples on the branches of the plot.

Fig. 4(A) presents the HCA dendrogram of Raman spectral data from *R. damascena* essential oil samples (n = 12) and commercial samples (n = 20). Authentic samples and commercial samples were clustered into two main groups (numbered as "1″ and "2"). Authentic *R. damascena* essential oil samples were located on the left branch (numbered as 1), and fraudulent samples were located on the right branch (numbered as 2) of the HCA dendrogram. As can be seen, authentic *R. damascena* essential oil samples were classified separately from commercial samples except for the samples coded as T1 and T5. One can understand from these results that only two of the commercial samples were authentic since these samples were clustered between *R. damascena* essential oil samples. The rest of the commercial samples were fraudulent or imitated samples. The HCA dendrogram clearly visualized the clusterization pattern of investigated samples.

Additionally, PCA was applied to the Raman spectra of investigated samples. Fig. 4(B) presents the PCA results (PC1 vs PC2) for the spectral range of 2000–200 cm^{-1}. Here, PC1 explained 54.56% of the total variance among samples. Further, PC2, PC3, PC4, PC5, PC6 and PC7 described the 17.03%, 12.03%, 5.01%, 4.55%, 3% and 1.83% of total variance, respectively. Eigenvector plots of the PCA are presented in the supplementary file (S1). A clear classification pattern was observed between all samples. Authentic samples (R1-R12, T1, T5) were marked with green squares and fraudulent samples were marked with red triangles. Only two of the commercial samples (T1 and T5) were located between authentic samples in the 2-D PCA plot of Raman spectra. The first two PCs explained the 71.6% of the total variance among samples. Obtained results were 100% compatible with those obtained by the HCA .The results showed that PCA findings confirmed those obtained by HCA.

Briefly, PCA and HCA were performed for discrimination and identification of *R. damascena* essential oil samples based on Raman data. Obtained findings confirmed those obtained by HCA and PCA analyses of FTIR data from all samples.

3.5. Determination of the volatile composition of R. damascena essential oil

In the present research study, *R. damascena* essential oil (n = 12) and commercial samples (n = 20) were evaluated using the GC–MS technique. Table 1 presents the volatile aroma compounds identified in Turkish *R. damascena* essential oils through GC–MS analysis. A total of 38 compounds were identified in twelve *R. damascena* essential oil samples. Detected and identified compounds represented the 96.89% of overall essential oil composition. Twelve *R. damascena* essential oil samples (R1-R12) presented the same chemical composition with similar identification results; thus, the volatile profile of R1 is presented in Table 1. The percentage compositions (area %) of the identified volatile compounds are presented in Table 1. The volatile compound profile of *R. damascena* essential oil was composed of monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, hydrocarbons and various oxygenated compounds. Major compounds were determined as citronellol (34.00%), geraniol (18.00%), nonadecene (11.42%) and nerol (7.9%). Certain characteristics of *R. damascena* cultivated in Turkey, Morocco and Bulgaria were specified in the ISO 9842–2003 standards (ISO, 2019). Detected percentages of ethanol (1.18%), citronellol (34.00%), nerol (7.9%), geraniol (18.00%), phenyl ethyl alcohol (1.40%), heptadecane (2.1%), nonadecane (11.42%) and heneicosane (3.91%) were in compliance with those reported in ISO 9842–2003 standards. The most abundant volatile compound was determined as citronellol. Prior studies have noted the importance of citronellol in the development of the main rosaceous character of rose oil. Citronellol, geraniol and phenyl ethyl alcohol contents were reported to be major criteria for the determination of the commercial quality of *R. damascena* essential oil (Toluei et al., 2019). The *R. damascena* oil quality is evaluated by the citronellol/geraniol ratio, and it was reported that this ratio is preferred to be between 1.0 and 1.5 (Nedeltcheva-Antonova et al., 2017). Previous studies reported that citronellol/geraniol ratios were determined as 2.07, 2.34, and 2.07 for Bulgarian, Turkish and Iranian essential oils, respectively (Pellati et al., 2013). A previous study reported that the citronellol/geraniol ratio of Iranian *R. damascena* was determined as 3.8. This value may alter due to geographical and climatic conditions. In the present research, the citronellol/geraniol ratio was determined as 1.89. In the current research, heptadecane, methyl eugenol, farnesol isomer B and 1-nonadecene were observed at the relative concentrations of 2.1%, 2.15%, 1.18% and 3.24%, respectively. Relative percentages of other constituents were determined to be lower than the concentration of 1%.

Table 1
Volatile aroma compounds identified in Turkish *Rosa Damascena* essential oils by GC–MS analysis.

RI[a]	Content (%)	Chemical Name	Chemical Formula
	1.18 ± 0.25	Ethanol	C_2H_6O
869	0.19 ± 0.04	1-hexanol	$C_6H_{14}O$
931	0.91 ± 0.08	α-Pinene	$C_{10}H_{16}$
968	0.20 ± 0.03	Heptanol	$C_7H_{16}O$
972	0.05 ± 0.01	Sabinene	$C_{10}H_{16}$
976	0.22 ± 0.05	β-Pinene	$C_{10}H_{16}$
993	0.36 ± 0.08	β-Myrcene	$C_{10}H_{16}$
1102	0.79 ± 0.06	Linalool	$C_{10}H_{18}O$
1115	0.15 ± 0.02	*cis*-Rose oxide	$C_{10}H_{18}O$
1117	1.40 ± 0.34	Phenylethyl alcohol	$C_8H_{10}O$
1128	0.08 ± 0.02	trans-Rose oxide	$C_{10}H_{18}O$
1180	0.35 ± 0.08	4-Terpineol	$C_{10}H_{18}O$
1191	0.24 ± 0.08	α-Terpineol	$C_{10}H_{18}O$
1220	34.00 ± 0.55	Citronellol	$C_{10}H_{20}O$
1238	0.89 ± 0.08	*cis*-Citral	$C_{10}H_{16}O$
1246	7.9 ± 0.16	Nerol	$C_{10}H_{18}O$
1263	18.0 ± 0.85	Geraniol	$C_{10}H_{18}O$
1278	0.2 ± 0.02	*trans*-Citral	$C_{10}H_{16}O$
1360	0.51 ± 0.07	Citronellyl acetate	$C_{12}H_{22}O_2$
1365	0.78 ± 0.04	Eugenol	$C_{10}H_{12}O$
1395	0.2 ± 0.05	β-Elemene	$C_{15}H_{24}$
1401	2.15 ± 0.35	Methyl eugenol	$C_{11}H_{14}O_2$
1416	0.8 ± 0.01	*trans*-Caryophyllene	$C_{15}H_{24}$
1449	0.38 ± 0.03	α-Humulene	$C_{15}H_{24}$
1456	0.32 ± 0.04	α-Guaiene	$C_{15}H_{24}$
1481	0.81 ± 0.04	Germacrene D	$C_{15}H_{24}$
1500	0.31 ± 0.03	Pentadecane	$C_{15}H_{32}$
1504	0.06 ± 0.01	Azulene	$C_{10}H_8$
1600	0.02 ± 0.02	Hexadecane	$C_{16}H_{34}$
1695	1.18 ± 0.27	Farnesol isomer B	$C_{15}H_{26}O$
1700	2.1 ± 0.16	Heptadecane	$C_{17}H_{36}$
1713	0.11 ± 0.04	*cis*-Farnesol	$C_{15}H_{26}O$
1900	11.42 ± 0.18	Nonadecane	$C_{19}H_{38}O_2$
1905	0.16 ± 0.01	2-methyl-7-nonadecene	$C_{20}H_{40}$
1931	3.24 ± 0.36	1-Nonadecene	$C_{19}H_{38}$
2000	0.92 ± 0.06	Eicosane	$C_{20}H_{42}$
2100	3.91 ± 0.74	Heneicosane	$C_{21}H_{44}$
2300	0.4 ± 0.07	Tricosane	$C_{23}H_{48}$
Total	**96.89**		

[a] Relative Retention Indices relative to *n*-alkanes (C8–C40) on Rtx-5MS fused silica capillary column (30 m × 0.25 mm (internal diameter) 0.25 μm).

3.6. Determination of the authenticity of R. damascena essential oil based on GC–MS data

The total ion chromatograms (TICs), which is built by summing up intensities of all mass spectral peaks belonging to the same scan, were collected for *R. damascena* essential oil samples (n = 12) and commercial samples (n = 20). A TIC is composed of the total ion signal of a series of mass spectra, which are recorded as a function of chromatographic retention time. In other words, TICs include the whole information from the entire data set of samples. Here, HCA was applied to the TIC data of

all samples by SIMCA 15 (Umetrics, Umea, Sweden). As mentioned in previous sections, HCA reveals the intrinsic relationship between investigated elements. Additionally, the HCA dendrogram provides the opportunity to observe intercorrelations among the different samples. Fig. 5(A) displays the HCA dendrogram (Ward's algorithm) of GC–MS TICs from *R. damascena* essential oil samples (n = 12) and commercial samples (n = 20). As can be seen from the dendrogram, thirty-two samples (n = 32) were divided into three main clusters. Authentic *R. damascena* essential oil samples were clustered in the same branch (numbered as 3). The sample T20 (commercial sample) was clustered separately from all samples. Test samples T1 and T5 were clustered among the *R. damascena*; thus, one can conclude from this finding that T1and T5 were *R. damascena*. Additionally, predicted scores of PCA are presented in Fig. 5(B). All samples and PCA scores are visualized by the SIMCA score plot. The HCA results of the GC–MS findings confirmed those obtained by HCA of Raman and FTIR spectroscopy. Two of the commercial samples were determined to be authentic *R. damascena* essential oil samples. Results presented that eighteen commercial samples included impurities or additional substances in their compositions.

4. Discussion

R. damascena essential oil is a high-value natural product with considerable economic importance. Tracing the quality of *R. damascena* essential oil has great importance throughout the whole world. Considering the necessity for the development of new, effective, reliable, strong and low-cost analytical techniques for determination of the authenticity of essential oils, there is a growing interest in innovative analytical methodologies combined with multivariate chemometrics (HCA & PCA). The current research characterized the *R. damascena* essential oil using FTIR, Raman and GC–MS techniques. Results showed that FTIR, Raman and GC–MS techniques combined with chemometrics (HCA & PCA) could be used effectively for the determination of the authenticity of *R. damascena* essential oil. Authentic *R. damascena* were clearly discriminated from fraudulent commercial samples with

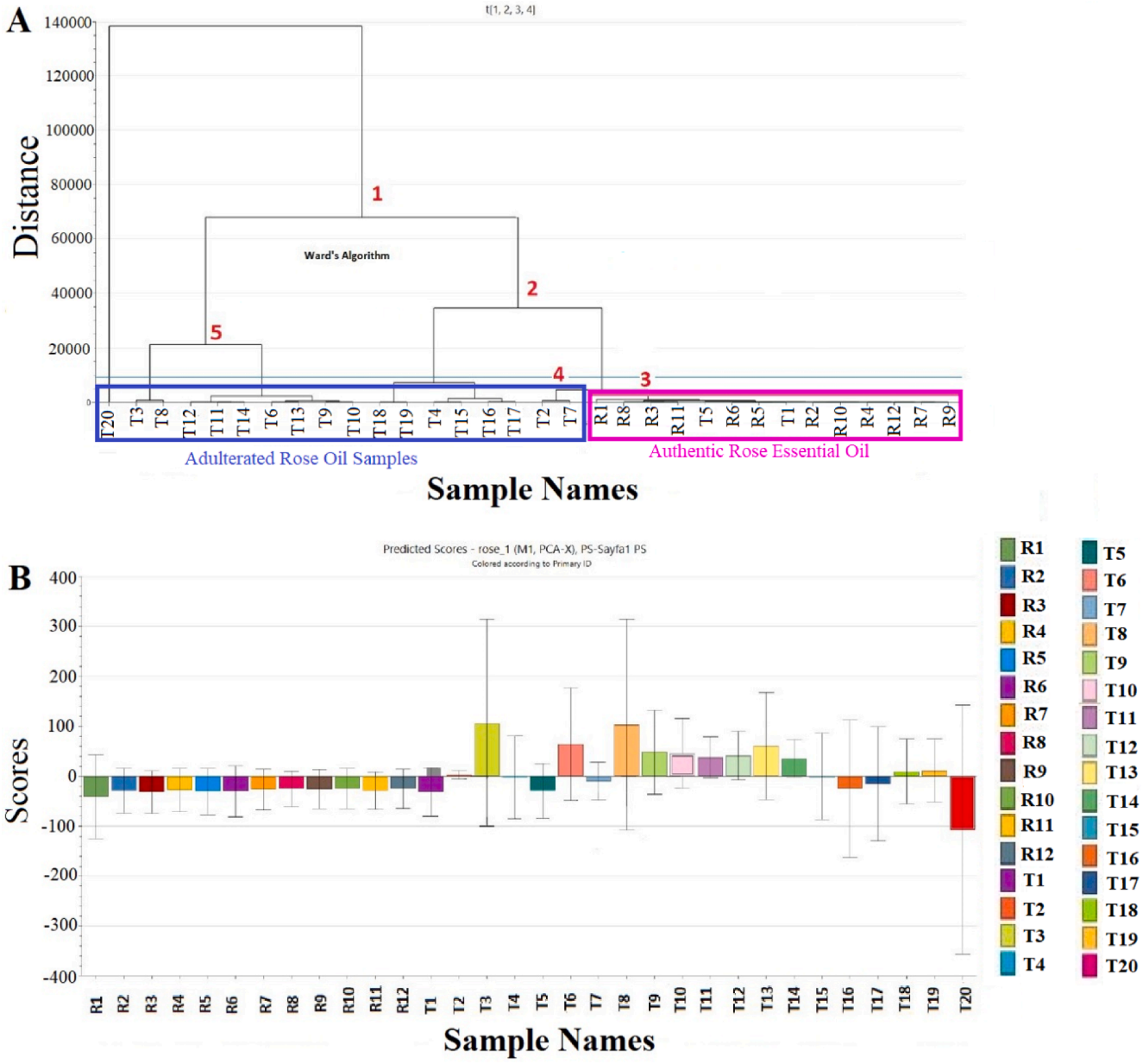


Fig. 5. (A) HCA dendrogram (Ward's algorithm) of GC–MS total ion chromatograms from *Rosa damascena* essential oil samples (n = 12) and commercial samples (n = 20). (B) Predicted scores plot of PCA analyses (SIMCA).

minimum sample preparation. To the best of our knowledge, this is the first time that FTIR and Raman techniques combined with chemometrics have been used for the evaluation of the authenticity of *R. damascena* essential oil. Clear classification of all samples with 100% accuracy was obtained using HCA and PCA. In particular, HCA dendrograms provided a visual presentation of the intrinsic relationship between authentic and commercial samples through main clusters and sub-clusters. The success of vibrational spectroscopy combined with chemometrics for determination of the authenticity of essential oils was proved in various previous studies. Some researchers have presented the successful results for natural products such as thyme, oregano, chamomile, eucalyptus and lavandin essential oils (Baranska et al., 2006; Bombarda et al., 2008; Schulz et al., 2003). In the current research, results from FTIR and Raman techniques confirmed those obtained by each technique. The same classification pattern and clusters were observed in the chemometrics of both FTIR and Raman techniques. Only two commercial samples (T1 and T5) were determined as authentic *R. damascena* essential oil. Ward's algorithm was used in the chemometrics of FTIR, Raman and GC–MS techniques. The success of the chemometrics can be attributed to utilizing Ward's algorithm. Ward's algorithm HCA classification procedure is built on big data and is not limited to classification problems (Cebi, Yilmaz, & Sagdic, 2017). Ward's minimum variance method joins at each stage of the cluster pair whose merger minimizes the increase in the total within-group error sum of squares based on the Euclidean distance between centroids (Murtagh & Legendre, 2011). Previous studies have demonstrated that HCA analyses through Ward's algorithm were successfully employed to cope with various authenticity and adulteration problems (Cebi, Yilmaz, & Sagdic, 2017; Cebi et al., 2019; Muik et al., 2004; Nie et al., 2007; Ropodi et al., 2015; Taylan et al., 2020). Additionally, previous studies have reported that vibrational spectroscopy was successfully employed for determination of the adulteration and authenticity in various oil food products (de Lima et al., 2020; Mandrile et al., 2016). Briefly, current findings and previous contributions have shown that vibrational spectroscopy combined with chemometrics has great capability to provide accurate and reliable results for determination of the authenticity of essential oils such as *R. damascena* essential oil.

5. Conclusion

The current research evaluated the capability of FTIR, Raman and GC–MS techniques combined with chemometrics for discrimination of authentic *R. damascena* essential oil from fraudulent commercial samples. HCA and PCA analyses were successfully employed for observation of the classification pattern of authentic and fraudulent samples. All of the applied analytical techniques (FTIR, Raman and GC–MS) distinguished authentic *R. damascena* essential oil samples using chemometrics. As result, two of the commercial samples (T1 and T5) were determined as authentic *R. damascena* essential oils since they were located between authentic samples in the HCA and PCA plots.

To the best of our knowledge, this study is the first to determine authenticity of *R. damascena* essential oil using FTIR, Raman and GC–MS techniques in combination with chemometrics. One of the strengths of this study is that it represents a comprehensive application of three robust analytical techniques. GC–MS results confirmed those obtained by FTIR and Raman methodologies. Further, FTIR and Raman techniques combined with chemometrics of HCA and PCA may be used for fast, economic, reliable, easy and non-destructive analysis of *R. damascena* essential oils for authenticity control. It is recommended that further research be undertaken in the detection and quantification of potential adulterants such as palmarosa essential oil, geraniol essential oil, benzyl alcohol, benzyl benzoate, dipropylene, dipropylene glycol, diethyl phthalate and synthetic compounds in the composition of *R. damascena* essential oil using strong analytical techniques.

CRediT authorship contribution statement

Nur Cebi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Muhammet Arıcı:** Conceptualization, Methodology, Writing - review & editing, Visualization, Supervision. **Osman Sagdic:** Conceptualization, Methodology, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129495.

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