Real-time Quality Authentication of Honey Using Atmospheric-Pressure Chemical Ionization Mass Spectrometry (APCI-MS)

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Abstract

The aim of this study was to use gas chromatography-mass spectrometry (GC-MS) and APCI-MS techniques to detect adulteration in honey. The key volatile compounds in the headspace of the adulterated honeys were marked by GC-MS and their representative fragment ions were utilized in scanning honey samples using the real-time APCI-MS system. The PLS models validated using independent datasets resulted in coefficient of determination (R_p^2) of 0.97 and 0.96 and root mean square error in prediction (RMSEP) of 2.62 and 2.45 for the GC-MS and APCI-MS datasets, respectively. The most efficient volatiles from GC-MS analysis and their corresponding fragment ions *m/z* from APCI-MS data analysis were then identified and used to develop new PLS models to predict the level of adulteration. The best PLS model gave R_p^2 of 0.95 and RMEP of 2.60% in the independent validation set indicating that the model was very accurate in predicting the level of adulteration.

***Keywords**: Volatile compounds, aroma, honey, GC-MS, APCI-MS, adulteration, headspace, PLS*

Introduction

Honey is a natural sweet substance produced by *Apis mellifera* bees from nectar and secretions of flowering plants or the excretions of plant sucking insects on the surface of the plants (CODEX Standard 12, 2001). It consists of a mixture of sugars (mostly glucose and fructose) and water in addition to various amounts of other substances including proteins, enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, phenolic compounds, pigments, pollen and volatile aromatic compounds (Ciulu et al., 2011; Alqarni et al., 2012; Escuredo et al., 2013) with many nutritional and medical merits (Pontes et al., 2007). The flavour of honey is composed of a complex blend of many compounds, including alcohols, aldehydes, ketones, esters, lactones, sulfides and free fatty acids.

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The key factors that determine the overall quality of honey in terms of composition, colour, aroma, taste and flavour depend mainly on the floral source of nectar, geographical regions, seasonal conditions, environmental factors and honeybee species involved in its production in addition to the beekeeping practices during production, processing, packaging, handling and storage (Tornuk et al., 2013; Escuredo et al., 2014). Honey may change and degrade over time, due to natural enzymes, high temperature and extended storage time, this may lead to the formation of new components such as furans, amino acids, alcohols, phenolic compounds and new volatile compounds (da Silva et al., 2016). These changes may be detrimental to the sensory quality of honey, but are acceptable within the standard definition of honey as a natural substance; however this could be violated through the addition of different foreign substances (Fuente et al., 2011). According to the regulations outlined by the Codex Alimentarius standard (CODEX Standard 12, 2001) and the EU Honey Directive 2001/110/EC (Council Directive, 2002), any practices of adding or removing any ingredients or substances from the natural pure honey that may affect its composition, flavour, taste and aroma are strictly prohibited. This issue of honey fraud is a growing critical problem due to its negative impacts on consumer health, nutritional status and fair trading practices.

Owing to the limited production levels, limited availability and the relatively high price of honey, the issue of honey fraud is very obvious in various forms. The easiest form of honey tampering is diluting honeys with water, adding inexpensive sweeteners (e.g. inverted sugar syrups, corn syrups, high fructose or maltose syrup) or indirectly by feeding the honeybees with sugar syrup (Perez-Arquillué et al, 1994; Puscas et al., 2013). Marketing low-quality honey as a high-quality honey or intentionally mislabelling the geographic location or the botanical origin of honey is another form of severe adulteration practices used by some unscrupulous suppliers to increase their profit margins. In general, when the product is not a pure honey, it is not allowed to be labelled as "Honey".

In many cases, adulteration of honey is rather difficult to detect owing to the diversity in the composition and physicochemical properties of different honeys collected from different botanical sources and geographical locations, and the similarity in chemical composition of added adulterants and the honey (Ruiz-Matute et al, 2010). Therefore, using only one property is sometimes not enough to evaluate the authenticity of all kinds of honey. For instance, a dark colour could be a sign of the botanical or geographical origin of a honey but also it could a sign of the storage conditions or a sign of heat treatment practised on the pure honey to inhibit or retard the crystallization process, or to block the development and growth of micro-organisms (Gámbaro et al., 2007; Vaikousi et al., 2009). Similarly, 5-Hydroxymethylfurfural (5-HMF) content, formed by the decomposition of monosaccharides or the Maillard reaction, could be used as indicative of honey deterioration due to heating or storage for a long time in unfavourable conditions or as a sign of falsification by adding inverted syrup (Capuano & Fogliano, 2011; Yücel & Sultanoglu, 2013). However, this compound cannot be used alone to determine the severity of the heat treatment, because some other factors such

as the sugar profile, presence of organic acids, pH, moisture content, water activity and floral source may affect its formation as well. In addition, 5-HMF can also be formed at low temperatures, even under acidic conditions, via subsequent dehydration reactions of sugars. Thus, the validity of 5-HMF as the only adulterant indicator is therefore questionable (Perez-Arquillué et al., 1994).

The authenticity of honey can be checked by a range of analytical methods to detect the fraud. These methods should directly look for the presence of expected compounds with definite concentrations (which distinguishes a certain honey from another) and to look for the presence of any unexpected compounds (which distinguishes a certain adulterant in the pure honey). There are many techniques utilized by researchers for detecting honey fraud based on chromatographic methods or non-chromatographic methods such as NIR spectrometry, nuclear magnetic resonance (NMR) spectroscopy, simultaneous distillation–extraction or microscopic detection techniques (Perez-Arquillué et al, 1994; Anklam, 1998; Jasicka-Misiak et al., 2012; Lenhardt et al., 2014; Siddiqui et al., 2017; Wu et al., 2017). Each of these techniques has its own advantages and limitations. However, methods routinely applied in the honey trade are relatively time-consuming and require tedious preparation of the samples as well as complex analytical equipment (Cozzolino et al. 2011). Therefore, there is an urgent need from researchers and regulatory authorities for the development of a new, rapid, simple, non-destructive, economical and reliable analytical procedure for the effective authentication of honey.

One of the most promising methods in honey authentication is the ion chromatography technique that depends on extracting and analysing the headspace aroma-related volatile compounds (Bertelli et al., 2008; Papotti et al., 2009; Manyi-Loh et al., 2011; Campillo et al., 2012; Kus et al., 2013). Advances in headspace chromatography techniques have reached an unprecedented level of development and a plethora of applications for food composition analysis and detection of adulteration and other forms of food fraud have recently been investigated. The term “headspace” refers to the gas phase above the honey sample placed in a closed vial sealed with a septum. The volatile compounds entrapped in the headspace that characterize one honey from another include aldehydes, ketones, acids, esters, alcohols, hydrocarbons, norisoprenoids, terpenes, benzene derivatives, furan, pyran and sulfur compounds (Radovic et al., 2001; Manyi-Loh et al., 2011; Bentivenga et al., 2004). Nonetheless, these compounds are originated basically from plant nectar, transformation of plant compounds directly by honeybees, generated by heating or enzymatic treatment during honey processing and storage, or from microbial or environmental contamination (Castro-Vázquez et al., 2007; Cuevas-Glory et al., 2007; Jerković & Marijanović, 2009). Thus, they represent a unique fingerprint of a specific honey that could be used to discriminate one monofloral honey from another and provide the required information about the botanical and geographical origin of such honeys. Nevertheless, using these fingerprints in tandem with the relevant chemometric methods seems to have more potential than the use of single markers as used by the majority of other analytical methods. Headspace analysis is quite

simple and comprises of a sealed vial containing the honey sample. The headspace volatiles can be directly trapped using gas-tight syringes or other devices based on various trapping materials such as solid-phase microextraction (SPME) and single-drop microextraction (SDME). The SPME fibre provides an excellent sorption capacity and will extract a broad representative range of volatiles from the headspace of the honey (Čajka et al, 2007). However, requirement of standardized extraction conditions besides the prolonged time required for extraction and analysing the data represent constraints of employing this technique in expeditious real-time applications. In this regard, APCI-MS has been implemented successfully in real-time tracking of aroma-related volatile compounds released from food stuffs to evaluate quality changes during different processing regimes (Linforth et al., 1999; Taylor et al., 2000; Fisk et al., 2011; Fisk et al., 2012). Therefore, this technique can be used to meticulously evaluate the volatile profile of honey with minimum sample preparation to monitor the presence or loss of characteristic volatile compounds in the sample analysed. Thus, the aim of this work was to utilize headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME/GC-MS) to identify and semi-quantify the major volatile compounds in Egyptian honeys for the purpose of detecting adulteration with inverted sugar syrup. Subsequently, a real-time direct injection headspace atmospheric-pressure chemical ionization-mass spectrometry (HS/APCI) protocol was then developed to target specific predefined key fragment ions to quantify the concentrations of target adulterants in honey samples with the aid of chemometric multivariate analyses. To the best of our knowledge, this is the first study devoted to characterize the volatile compounds in Egyptian honeys of different floral sources. The study also highlights the potential of volatile compounds as markers of adulteration in these honeys and illustrates a novel real-time technique for their detection using headspace GC-MS and APCI-MS.

Materials and Methods

Preparation of pure and adulterated honey samples

Pure honeys from four different floral sources: Citrus (*Citrus spp.*), Alfalfa (*Medicago sativa*), Marjoram (*Origanum majorana*) and Black seed (*Nigella sativa*) were purchased from private apiaries in Egypt who guarantee their initial authenticity. All honey samples packaged in glass jars have not been undergone any treatment that could alter their composition before testing. Prior to analysis in either GC-MS or APCI-MS equipment, a diluted solution of each honey sample was prepared by adding MilliQ deionized water with a ratio of 5:1 (v/w), vortexed for 10 min and sonicated for 30 min until a homogenised clear solution was achieved. Exactly 8 ml from the diluted honey was placed into a 15 ml amber glass vial and 20 µL of an internal standard (ISD) was added to each vial. The ISD was prepared by adding 10 µL 3-heptanone (Sigma, Saint Louis, MO) into 10 mL methanol (Laboratory reagent grade; Fisher Scientific, Loughborough, UK). The vials were then

hermetically sealed with a magnetic cap with PTFE/silicon septum for SPME extraction. All analytical samples were randomised for GC-MS analysis. In this study, inverted sugar syrup was used as an adulterant. Counterfeit honeys were prepared by adding different concentrations of the syrup (3 – 39 % at 3% intervals) to the pure honey to intentionally simulate honey adulteration at different levels. These levels of adulteration were chosen to examine the ability of both GC-MS and APCI-MS systems in tandem with the devolved PLS models in predicting the amount of the added adulterant from very low concentration (3%) to a very high concentration (39%). The adulterated honey was then diluted with MilliQ deionized water using the same procedure. A total of 136 pure and adulterated honey samples with different concentrations of syrup were prepared and stored at 4°C until analysed. The key steps involved in the whole procedure of detecting adulteration starting from sample preparation, optimization, headspace extraction/analysis on GC-MS and APCI-MS, data analyses and modelling are shown in Figure 1.

Extraction of honey volatiles using solid phase micro extraction (SPME)

Headspace solid phase micro extraction coupled to gas chromatography–mass spectrometry (HS-SPME/GC-MS) was used to extract and analyse the volatile compounds from honey samples. Before analysis, the solid phase micro extraction (SPME) fibre (Carboxen Polydimethylsiloxane fibre, Supelco, Sigma Aldrich, UK) was preconditioned in the injection port of the gas chromatograph system according to the instruction provided by the manufacturer (60 min at 270°C). The GC-MS was supported with a preprogramed robotic SPME sampling unit (CombiPal. Zwingen, Switzerland) to automatically control the conditioning, extraction and injection processes. The SPME has a 2-cm length StableFlex fibre with 50/30 µm divinylbenzene/Carboxen on polydimethylsiloxane coating (DVB/CAR/PDMS) to trap all possible volatile compounds in the headspace. After completing the extraction step, the SPME fibre was retracted from the vial and inserted into the injection port of the GC–MS where the volatile compounds were thermally desorbed for 2 min and transferred directly to the analytical column. A Trace GC Ultra (Thermo Scientific, Waltham, MA, USA) attached to an TSQ series mass spectrometer (Thermo Scientific Waltham, MA, USA) was used to analyse the volatiles in electron ionisation mode with ion source temperature of 200°C and a scanned mass range of m/z 30–300. The volatile compounds were separated in the GC equipment using a ZB-Wax fused silica capillary column (100% polyethylene glycol phase, 30 m, 0.25 mm, 1.0 µm; Phenomenex, Torrance, CA). The GC oven was held at 40°C for 3 min then heated up to 160°C at 4°C/min, raised to 200°C at 10°C/min, raised to 230°C at 125°C/min and then maintained constant at this temperature level for 5 min. Helium (at 99.999% of purity and at 1.5 bar) was the carrier gas with a constant flow rate of 1.0 ml/min in splitless mode. Also, blank analyses using empty vials without samples were run in order to characterise possible contaminants from the fibre or from the chromatographic system. Volatile compounds were identified by comparing their experimental retention times and mass

spectral fragmentation patterns with pure standards and those reported in the mass spectral library (NIST/EPA/NIH Mass Spectral Library, version 2.0; National Institute of Standards and Technology, Gaithersburg, MD). The identification of volatile compounds was confirmed by calculating Kovats linear retention indices (RI). Thus, a homologous series of n-alkanes with a chain length from C6 to C40 (Sigma-Aldrich Ltd., Dorset, UK) was injected under the same chromatographic conditions described above and used for determining the retention indices (RIs) of all detected volatile compounds. Hence, they were compared with literature values to support such tentative identification (Adams, 2007; Bianchi et al., 2007; Soria et al., 2009; Plutowska et al., 2011; Karabagias et al., 2014). By this way, the joint use of mass spectrometric data and RIs helps in providing a more assured identification of the detected volatile compounds (Bianchi et al., 2007). The semi-quantification of all volatile compounds (their estimated concentration in μg g^{-1}) was obtained directly from their integrated peak areas against the peak area of the internal standard.

Optimization of the extraction process

It is well known that several factors, including conditioning time, extraction time, extraction temperature, desorption time, ionic strength, amount of sample, sample/water ratio, sample solution/headspace volume ratio, and the type of fibre affect the performance of HS-SPME in recovering the volatile compounds from the sample headspace. The purpose of optimization was to select the ideal extraction conditions that provide the best extraction yield and minimize fibre malfunction and saturation. By using the Design Expert Software (Stat-Ease Corp., Minneapolis, MN), different levels of conditioning time (t_{cond}: 20–60 min), extraction temperature (T_{ext}: 50–70°C) and extraction time (t_{ext}: 20–60 min) were optimized using central composite design (CCD, with $\alpha = 1.682$) based on a 2^3 full factorial experiments, plus six axial points and six replicates in the centre of the domain. These experiments were performed in triplicate and conducted in a randomized order. To optimize these three variables simultaneously, one single criterion called 'desirability' was used to evaluate their responses in terms of the global peak area of all volatile compounds detected in the chromatogram (Bertelli, et al., 2008). The values of the optimal operating conditions that maximize the value of desirability were defined as the "Optimal Point" and were then selected and used for all subsequent analyses. To ensure that the final "optimal point" is valid for extracting volatile compounds from each kind of the unifloral honey examined in the study, a multifloral honey from a combination of the four unifloral honeys was used to optimize the HS-SPME extraction parameters by homogenously mixing equal amounts from the four unifloral honeys in one jar. ~~Moreover, the effect of different sample dilutions (1:1, 3:1 and 5:1 w/v) was also tested in order to avoid problems related to sample viscosity and to obtain reproducible results.~~ To avoid problems related to sample viscosity and to obtain reproducible results during extraction, all pure honey samples were first diluted with MilliQ water with a ratio of 5:1 before testing in the HS-SPME/GC-MS or the APCI-MS systems

(Plutowska et al., 2011). ~~The preliminary experiment indicated that the ratio of 5:1 (honey: water) of sample dilution showed good reproducibility and precision.~~

Extraction on the APCI-MS system

APCI-MS supported with an MS nose interface (Micromass, Manchester, UK) and fitted to a Quattro Ultima mass spectrometer (Waters Corporation, Milford, MA) was used for the static headspace analysis of honey samples by monitoring the ions of mass to charge (m/z) ratios from 30–300. The intensity of these fragment ions was measured at a cone voltage of 20 V, source temperature of 75°C and dwell time of 0.5 s. Exactly 15 ml aliquots of either pure or adulterated diluted honey were placed inside a glass screw-top vial and hermetically sealed with its tighten cap for headspace analysis. Similar to the incubation conditions used during the GC-MS analysis, each sample was held in a temperature controlled water bath (Precision, Jouan Inc. Winchester, Virginia, USA) at 70°C for 30 min before measuring the volatiles to allow equilibration of the volatiles released from the honey samples into the headspace. In practice, the static headspace above the sample was drawn through the MS nose interface into the APCI-MS source at a rate of 30 mL/min and then analysed in the full scan mode. All analyses were run in triplicate and the three readings were averaged for each sample.

Data Analysis

Acquisition of total ion chromatograms in GC-MS system, collection of mass spectra, library search and peak deconvolution were performed using Thermo Scientific™ Xcalibur™ Software (Thermo Scientific, Waltham, MA, USA) to calculate the peak areas and relative concentrations of volatile compounds found in the headspace; whereas the mass spectra from APCI-MS dataset were exported using Waters Masslynx™ Software version 4.1 (Waters Corporation, Milford, MA, USA) to determine the intensities of the dominant fragment ions having different *m/z* ratios found in the headspace. Pure and adulterated honey samples having different concentrations of the adulterant (n = 136) were divided into two data sets: the calibration set (n = 91) to be used for developing the chemometric-based calibration model and a prediction set (n = 45) to check the validity of such developed model in predicting the exact amount of the adulterant in the samples. Chemometric analyses using partial least squares (PLS) regression were carried out using the Unscrambler software (version 9.7, CAMO AS, Norway) under segmented cross validation scheme to predict the amount of the adulterant added to each honey sample. In segmented cross validation, samples were divided into subsamples and a single segment of five subsamples was then retained as a validation dataset for testing the model developed on the rest of the other subsamples. The cross-validation process was then repeated, with each of the five subsamples used exactly once as a validation dataset. The ideal number of latent factors of the best calibration PLS models were then identified at the minimum value of the predicted residual error sum of squares (PRESS) in order to minimize the risk of overfitting

(Cozzolino et al., 2008). All data were pre-treated first using the standard deviation scale in which the data for each variable (the volatile compounds in GC–MS dataset or the fragment ions *m/z* in APCI-MS dataset) was divided by its corresponding standard deviation prior to chemometric application to remove the drifts and baseline effects. Despite floral source of the honey, the main purpose of the PLS regression was to determine the fundamental relationship between multiple dependent predictor variables (the volatile compounds in GC–MS dataset or the fragment ions in APCI-MS dataset) and the amount of the adulterant in honey. Furthermore, the values of the model's loadings and the regression coefficients of the predictor variables were used as exploratory analysis tools to select the marker compounds most related to the honey adulteration. The analyses of PLS regression coefficients unravel the fragments (*m/z*) responsible for classification of honey samples based on the amount of the adulterant present (Aliferis et al., 2010).

Results and Discussion

Optimization of extraction method

The central composite design (CCD) carried out to select the ideal operating conditions of the HS-SPME/GC-MS. Three factors were evaluated: conditioning time (t_{cond}: 20–60 min), extraction temperature (T_{ext}: 50–70°C) and extraction time (t_{ext}: 20–60 min). The design required a total of 20 experiments including 2^3 = 8 full factorial experiments, 6 experiments for axial levels and 6 experiments for the central points. The design allows the evaluation of the individual effects of these three factors as well as the two- and three-order interactions among them. These combinations of experiments were conducted three times and the average peak area was taken as the response variable. The best experiment, corresponding to the optimum levels of these three factors, was defined where the highest signal intensities (largest peak areas) of all detected volatile compounds in the chromatogram were achieved. The interaction effects of extraction temperature and extraction time at different levels of conditioning time in terms of desirability function are illustrated in Figure 2. Although all of these three variables had influenced the desirability function, extraction time was most significant compared to the other two variables. At short extraction times (e.g. 20 min); increasing either conditioning time or the extraction temperature decreases the model desirability to less than 0.2. However, long extraction times (e.g. 60 min) substantially increased the model desirability in spite of the values of either extraction temperature or conditioning time. The desirability function in this zone was higher than 0.90. As shown in Figure 2, the best overall desirability of the design was obtained when the extraction temperature and extraction time were adjusted at their highest level (T_{ext}: 70°C min & t_{ext}: 60 min). Very little improvement was achieved when the conditioning time (t_{cond}) increased from 20 min to 60 min (from Figure 2A to Figure 2C), but this improvement was not significant. Accordingly, a 30 min conditioning time at extraction temperature of 70°C and a 60 min extraction

time was defined as the optimum setting to obtain a good design for the best extraction of volatile compounds in the honey samples. These findings are in a close agreement with that reported by Bertelli et al. (2008), Ceballos et al. (2010); Plutowska et al. (2011) and Robotti et al. (2017) in extracting volatiles from some unifloral and polyfloral honeys. These selected optimum values were then used to evaluate volatile compounds in honey samples for all subsequent analyses.

Volatile compounds in Egyptian honeys

Honey samples collected from Egyptian apiaries were all remarkably different from one another as illustrated in their GC-MS total ion chromatograms (TIC) shown in Figure 3. Even without complicated analysis, the difference in the profiles of volatiles for different honey types in terms of the intensity of GC peaks can be easily observed and all remarkable peaks in the chromatograms of the volatile profiles may be considered as characterising peaks to differentiate Egyptian honeys from different floral sources. By utilizing the developed optimized extraction protocol, a total of 119 different volatile organic compounds were detected, identified and quantified in the headspace of the pure Egyptian honeys by SPME-GC-MS: including 89 in citrus honey, 75 in alfalfa honey, 90 in marjoram honey and 87 in black-seed honey (Table 1 and Figure 3). The profile of volatile compounds of the honeys was found to be in accordance with those reported by several authors (Alissandrakis et al., 2007; Soria et al., 2009; Manyi-Loh et al., 2011; Kaškonienė & Venskutonis, 2010). These identified volatiles involved compounds from different chemical groups such as alcohols, phenols, ketones, organic acids, esters, aldehydes, aliphatic hydrocarbons, aromatic hydrocarbons, hydrocarbons cyclic (e.g. terpene like *D*-limonene). The calculated values of the retention indices (RI) of the identified volatile compounds shown in Table 1 were very close to those reported by Plutowska et al. (2011). Indeed, the monofloral honeys are never actually monofloral because the bees rarely collect nectar from the same floral source and may visit any type of flower they can reach (Kaškonienė & Venskutonis, 2010). Thus, the examined Egyptian honeys may be from overlapping floral sources. However, elucidation of the volatile organic compounds of a particular honey can help to standardize its quality and avoid fraudulent labelling of the product (Manyi-Loh et al., 2011). Among the 119 identified volatile compounds, only 62 compounds were found in all four examined honeys but their concentrations were markedly different from one honey to another as shown in Table 1. However, it is out of scope of this study to differentiate and identify the floral source of the examined honeys because the main task was to detect the adulteration with sugar syrup that may occur despite the floral source of the honey.

The volatile fraction composition in honey greatly depends on nectar composition and floral source. The citrus honey was characterised by having high concentration of *D*-limonene; furfural; dill ether (Anethofuran); β-Linalool; lilac aldehyde D; 3-Cyclohexene-1-acetaldehyde, α,4-dimethyl- and

methyl anthranilate (Nevoli oil) in addition to some unique volatiles such as trans rose oxide; 5-hepten-2-ol, 6-methyl- (Sulcatol) and 1,4-dimethyl-4-acetylcyclohexene. The potent volatile compounds in alfalfa honey were nonanal; furfural; nonanoic acid, methyl ester (*i.e.* Methyl nonanoate); decanal; 2-ethyl-hexanoic acid, and nonanoic acid besides 3-carene; 1-octen-3-ol; tetramethyl-pyrazine; 5-methyl-2-furancarboxaldehyde; 2,2'-bifuran and oxopholone were not detected in honeys from other floral sources. Egyptian honeys originated from Marjoram and black seeds have not been characterized before and this is the first study to investigate their volatile fraction composition. Marjoram honey is characterised by furfural; methyl nonanoate; benzaldehyde; β-linalool; benzeneacetaldehyde and nonanoic acid; meanwhile the most abundant volatile compounds found in honey originated from black seed were *D*-limonene; nonanal; furfural; 2-ethyl-1-hexanol; methyl nonanoate and benzaldehyde. Based on GC–MS data, furfural, nonanoic acid and 5-hydroxymethylfurfural were found in all tested honeys implying long storage periods or the high temperature during honey production (Agila & Barringer, 2013) and they are not necessarily to be markers of adulterations with syrup until reaching certain limits. Similar findings were reported by Radovic et al. (2001) who analysed 43 samples of honey from different countries (i.e. Denmark, Germany, Italy, France, Holland, Spain, Portugal and England) and found that the major volatile compounds detected by headspace analysis in such honeys were furfural, benzaldehyde and acetone.

By employing the same extraction routine, undecane; 5-methyl-2(3H)-furanone; furfural; 5-methyl-2-furancarboxaldehyde; 2-methyl-benzofuran; isomaltol; 2-(2-furanylmethyl)-5-methyl-furan; hepta-2,4-dienoic acid methyl ester; 2,5-furandicarboxaldehyde, 5-hydroxymethylfurfural and nonanoic acid were the key volatile compounds detected in the headspace of 'pure' sugar syrup samples (Table 2). It was observed that most of the substances identified in syrup headspace were also found in the samples of authentic honey (Table 1), which has negative implications for the possibility of using volatile profiles to detect this kind of adulteration. However, tracking the concentrations of these compounds could be the key parameter in detecting the presence of syrup in the counterfeit honey samples if it exceeds a certain limit.

Prediction of the adulteration level

The presence of key volatile compounds associated with the adulterant was the key driver to discover the level of honey adulteration. When the adulteration level increased in a honey sample, higher concentrations of these compounds are expected to be recorded in the form of larger peak areas in the chromatograms or higher ion intensities in the mass spectra at the fragment ions shown in Table 2. When compared to raw honeys the concentration of volatile compounds shown in Table 2 increased incrementally with increased adulteration. On the other hand, the other volatiles that had been previously reported as being common volatiles in raw honeys significantly decreased in concentration

by the effect of dilution caused by adding different amounts of the adulterant. In the PLS regression model, the 62 mutual volatile compounds (identified in all tested honeys) and the key volatiles of the adulterant (Table 2) were used as predictor variables (X-variables); meanwhile the amount of adulterant added to the samples was utilized as the response variable (Y-vector). Hence, the main aim of the PLS calibration modelling was to build a linear relationship between the volatile concentrations of the headspace data from GC–MS (X-variables) and the amount of the added adulterant (Y-vector). Partial least squares regression (PLSR) compresses the spectral data into orthogonal structures called latent variables/factors which describe the maximum covariance between X-variables and Y-vector (Geesink et al., 2003). The parameters used to evaluate the efficiency of the developed model were the number of latent factors (LF), coefficient of determination (R^2) and the root mean square error (RMSE) between the modelled and actual amount of the added adulterant. The best model should have high coefficient of determination and low root mean square error in calibration (RMSEC) and cross validation (RMSECV). Moreover, the model developed using the calibration dataset (n = 91 samples) was tested in an independent prediction dataset (n = 45) in which the best model should provide high coefficient of determination (R_p^2) and low root mean square error in prediction (RMSEP). The RMSEP indicates the absolute fit of the model to the data and is a good measure of how accurately the model predicts the response (the amount of the adulterant in the honey sample). Table 3 indicated that the PLS model developed for the GC-MS data was very accurate in predicting the amount of the adulterant with R_c^2 of 0.93 and RMSEC of 3.03% for calibration of and R_{cv}^2 of 0.90 and RMSECV of 3.61% under cross validation. As shown in Figure 4 and Table 3, when this model was used with the independent data set it provided R_p^2 of 0.93 and RMSEP of 2.97%. The values of RMSE in the training and validation data sets (3.03% and 2.97%, respectively) implied that the developed PLS model developed on GC-MS data was not accurate enough in predicting low level of concentration (< 3%). However, the overall accuracy of the model was reasonably acceptable in predicting the adulteration. Table 3 summarizes the results of the PLS model developed on GC-MS data using all the 62 mutual volatile compounds as well as the key volatiles of the adulterant (X-variables).

By using multivariate analysis, it was possible to highlight the specific importance of all variables involved in the modelling process. Therefore, to identify the most influential volatile compounds most related to the change occurred in honey samples due to adulteration, the PLS bi-plot of scores of honey samples (of different adulteration levels) and loadings of the variables (*i.e.* the volatile compounds) was created in the same plot as shown in Figure 5. The second principal component (PC2) accounted for 14% of the variance and showed separation between honey samples. In the score plot, proximity between samples reflects similarity in relation to their compositional features (Juan-Borrás et al, 2014). On the other hand, factor loadings for each compound provide an indication of the importance of such compound over the principal component (Cuevas-Glory et al., 2012; Tahir et al.,

2016). The first principal component (PC1) accounted for 84% of the variance in the dataset and showed a trend with increasing adulteration level from left to right. The loading plot reveals that certain volatile compounds are responsible for discrimination between samples receiving different levels of adulteration. Hence, it is very clear to observe that undecane, furfural, 5-methyl-2-furancarboxaldehyde and 5-hydroxymethylfurfural are tightly correlated with those samples that received high levels of adulteration at the right hand side of the plot. In fact, these compounds are the key compounds of this kind of adulterant as listed in Table 2. This finding indicates that the adulteration of honey with sugar syrup could be easily tracked by monitoring the abundance of these particular compounds in honeys. The higher the concentrations of these compounds in honey, the more likely of adulteration is expected.

Prediction of adulteration level by APCI-MS

The data used in predicting the adulteration level in chemometric analysis of GC-MS data were sourced from the relative concentrations of the identified volatile compounds; meanwhile the data to be analysed in APCI-MS were the extracted intensities of all possible fragment ions (*m/z*) from 30-300. Thus, a full mass scan was initially performed by monitoring all *m/z* ratios in the pure and adulterated honey samples. The obtained complete mass spectra (m/z values of all dominant ions) of all samples were carefully checked before any data processing and only those *m/z* variables found in all honeys but with different intensities were considered for chemometric analysis. Therefore, a subset of 80 *m/z* target variables/ions was used as predictor variables (X-variables) to predict the identity of a sample. The results of the PLS calibration model developed under this condition (Model I) shown in Table 3 and Figure 6a indicated that the level of adulteration could be predicted with R_c^2 of 0.98 and RMSEC of 1.88% for the calibration set and R_{cv}^2 of 0.96 and RMSEC of 2.40 % by cross validation with 5 latent factors. Testing such a model in an independent validation set indicated that the model was very accurate in predicting the level of adulteration with R_p^2 of 0.96 and RMEP of 2.52%. Compared with the PLS model developed on the developed on GC-MS data, the PLS developed on the APCI-MS data was more accurate and could be used safely in predicting low concentrations of the adulterant.

Selection of significant fragment ions

While the PLS regression model was developed using all fragment ions *m/z* in the scanned range, the prediction could be performed also by selecting only key *m/z* values. The individual masses (each single *m/z*) could also be evaluated to gain an insight into the chemistry that is driving the multivariate discrimination of pure and adulterated honeys. Thus, a certain number of fragments *m/z* corresponding to the major volatile compounds in adulterated honey samples should be selected to minimize interference from unknown compounds. Such fragments *m/z* must be carefully chosen because many

m/z are produced by several different volatiles. Only the most important fragments *m/z* having the greatest influence for the prediction of adulteration should be kept in the model. In this study, the weighted regression coefficient of each single fragment *m/z* resulted from the best PLS model was used as a sign to identify the importance of each single *m/z* in predicting the level of adulteration. Hence, a relationship between the *m/z* and their corresponding regression coefficients was then plotted and the fragment *m/z* having the highest weighted regression coefficient was considered an influential variable in prediction. The plot shown in Figure 7 provides an insight into the role played by each single fragment ion *m/z* based on their regression coefficient values. According to this plot, the peaks at fragment *m/z* 96, 97, 98 and 99 produced by specific volatile compounds such as furfural (*m/z*: 96 and 97), 5-hydroxymethylfurfural (*m/z*: 97), 5-methyl-2(3H)-furanone (*m/z*: 98 and 99), undecane (*m/z*: 99) and nonanoic acid (*m/z*: 98) allowed good prediction of the adulteration level practised on honey samples. In some previous studies carried out in selected ion flow tube mass spectrometry (Agila & Barringer, 2012 and 2013), some of these compounds such as furfural were reported to be very effective in detecting adulteration and identifying the floral sources of honeys.

Instead of using the whole range of fragment *m/z* (80 variables), a new PLS model (Model II) was developed using only these four ions *m/z* (96, 97, 98 and 99) as predictor variables. The results shown in Table 3 and Figure 6b revealed that such a model was very robust to accurately predict the level of adulteration with R_c^2 = 0.97 and RMSEC of 2.02% for the calibration set and R_{cv}^2 of 0.96 and RMSEC of 2.38 % for the cross validation scheme with 3 latent factors. Testing such a model with an independent validation set indicated that the model was very accurate in predicting the level of adulteration with R_p^2 of 0.95 and RMEP of 2.60%. From these results, it is easy to recognise that using only four fragment ions *m/z* has approximately the same efficiency in predicting the level of adulteration compared with using the full fragment ion *m/z* range.

In fact, instead of using a full scan mode to elucidate the most influential fragment ions, the volatile compounds resulting from analysing the GC-MS data (Figure 5) leads to the same conclusion. In other words, only the key fragment ions *m/z* from these volatile compounds, highlighted by GC-MS analyses, are required to discriminate between samples with different levels of adulteration. Accordingly, the major fragment ions *m/z* of undecane, furfural, 5-methyl-2-furancarboxaldehyde and 5-hydroxymethylfurfural could be directly used in a selected-ion mode in the APCI-MS system. Hence, it was clear that there was a kind of harmony between the results depicted in Figure 5 that shows the key volatiles responsible for detecting adulteration and those 'important' fragment ions *m/z* illustrated in Figure 7 obtained from APCI-MS analysis. Although the HS-APCI-MS analysis could not be used to unambiguously identify various aroma-related volatile compounds in honey samples like HS-GCMS analysis does, it provides an accurate estimation about the abundance of such compounds if their presence in the sample has been previously shown. One cannot assign a fragment ion *m/z* to a certain volatile compound from APCI-MS analysis alone unless it is confirmed by GC-

MS analysis because such ion could be a result of different forms of fragmentation of various volatiles. Thus, by knowing the key volatile compounds of an adulterant by GC-MS analysis, the assignment of fragment ions *m/z* to the corresponding headspace volatile compounds could be easily ascribed to the fragmentation patterns of this adulterant. The key fragment ions *m/z* highlighted by analysing the APCI-MS data (*m/z*: 96, 97, 98 and 99) indicated that the proposed method could be used directly in a real-time application for detecting adulteration based on quantitative assessment of these specific fragment ions.

Conclusion

The importance of honey quality authentication has recently increased because of problems associated with honey fraud negatively impacting market growth and damaging consumer confidence. Therefore, there is a critical need for the development of rapid, simple and precise tools for the detection of honey adulteration. In this study, the ability of headspace solid-phase microextraction with gas chromatography-mass spectrometry (HS-SPME/GC-MS) and atmospheric-pressure chemical ionization-mass spectrometry (APCI-MS) was tested for the rapid and accurate detection of adulteration of Egyptian honeys. Honeys from four different floral sources were subjected to adulterations with inexpensive sugar syrups of different concentrations (3-39%). The key volatile compounds were identified and quantified in the pure and adulterated honeys using HS-SPME/GC-MS and the PLS regression model developed on the whole volatile profile, these provided an accurate prediction of the adulteration level in honey samples (R_p^2 = 0.93 & RMSEP = 2.97%). Similarly, the PLS model developed on all fragment ions resulting from the APCI-MS analysis also gave accurate prediction of adulteration level (R_p^2 = 0.96 & RMSEP = 2.52%). The most influential fragment ions (m/z: 96, 97, 98 and 99) resulting from the analysis of APCI-MS data were identical to the fragment ions corresponded the same compounds that were identified by GC-MS. According to the comparison performed with our library, these fragments belong respectively to: undecane, furfural, 5-methyl- 2-furancarboxaldehyde and 5-hydroxymethylfurfural. The model developed using only these specific four fragment ions was very precise in predicting the level of adulteration (R_p^2 = 0.95 & RMSEP = 2.60%).

The suggested method could be easily used to recognise the identity of the honey and the presence of certain unexpected compounds in honeys such as sugar syrups. In essence, the ideal scenario should start first by identifying the key volatile compounds using GC-MS system and then utilize their corresponding fragment ions in selected-ion mode for real-time analysis on the APCI-MS system. To the best of our knowledge, this study is the first report that integrates the results of GC-MS with APCI-MS fingerprinting for Egyptian honeys and the detection of adulteration levels. Apart from the powerful prediction ability, the direct and robust nature of this suggested method makes it a very

promising technique in real-time authentication of various food products throughout processing regimes or during the handling chains.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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Legends to Figures

Figure 1 Key steps involved in detecting adulteration level in honey using headspace GC-MS and APCI-MS analyses.

Figure 2 Response surface plot for the desirability function versus extraction temperature (T_{ext}, °C) and extraction time (t_{ext} , min) at different values of conditioning time (A. t_{cond} = 20 min, B. t_{cond} = 40 min and C. t_{cond} = 60 min).

Figure 3 Typical total ion chromatogram (TIC) obtained by SPME of four unifloral Egyptian honeys (Citrus, Alfalfa, Marjoram & black seed) at the optimized extraction conditions.

Figure 4 Prediction of adulteration level in honey samples using PLS regression based on the concentration profiles of the headspace volatile compounds extracted by GC-MS. Actual versus predicted levels (%) of adulteration for calibration and validation sets.

Figure 5 Bi-plot of PLS sample scores and loadings of the volatile compounds (X-variables) along the first two principle components. The arrow indicates the direction of increasing the adulteration level.

Figure 6 Prediction of adulteration level in honey samples using PLS regression based on the fragment ion *m/z* profiles in the headspace extracted by APCI-MS. Actual versus predicted levels (%) of adulteration for calibration and validation sets using (a) full scan mode (Model I) and (b) selected ion mode '*m/z:* 96, 97, 98 & 99' (Model II).

Figure 7 Weighted PLS regression coefficients of all fragment ions *m/z* resulting from the model developed on the APCI-MS data. Circle highlights the most important ions *m/z* (*m/z*: 96, 97, 98 & 99).

Tables

Table 1 Retention time, retention index, characterizing ions and concentrations (in µg.g^{-1}) of all volatile organic compounds found in the Egyptian unifloral honeys from different floral sources.

Volatile Compound	RT (min)	RI*	Fragment Ions *m/z*	Botanical origin of the honey			
				Citrus	Alfalfa	Marjoram	Black
Acetaldehyde	1.91	711	44, 43, 42	0.0054	0.0032	0.0034	0.0019
Dimethyl sulfide	2.26	755	62, 47, 45	0.0063	0.0035	0.007	0.0061
Octane	2.62	765	114, 85, 43	0.0041	0.0078	0.0039	0.0023
Furan, 2-methyl-	4.01	883	82, 81, 53	0.001	0.0005	0.0011	0.0003
Nonane	4.3	887	128, 85, 57	0.0028	0.0047	0.005	0.0014
Butanal, 2-methyl-	4.94	924	86, 57, 41	0.0009	0.001	0.0027	0.0015
Butanal, 3-methyl-	5.06	928	86, 71, 58, 44	0.0005	0.0007	0.0015	0.0023
Ethanol	5.49	944	46, 45, 31	0.0812	0.0472	0.0471	0.0404
Furan, 2,5-dimethyl-	6.07	966	96, 95, 81	0.0011	0.0007	0.0012	0.0007
2-Methyl-2,3-divinyloxirane	6.84	994	110, 95, 67	0.0015		0.0004	0.0001
Decane	6.98	1000	142, 71, 57	0.0004	0.0009	0.0005	0.0002
Undecane	10.26	1095	156, 85, 71, 57	0.0251	0.0125	0.0221	0.0176
Hexanal	10.34	1097	100, 82, 56, 44	0.002	0.0019	0.0016	0.0021
1-Propanol, 2-methyl (Isobutanol)	10.6	1104	74, 43, 42	0.001	0.0007	0.0002	0.0009
β-Pinene	11	1115	136, 93, 69	0.0014			
2H-Pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-	11.14	1119	154, 139, 81, 71			0.0012	
Unknown (1)	11.78	1136	127, 72, 67	0.0008			
3-Carene	12.69	1160	136, 93, 91		0.0007		
β-Myrcene	13.28	1176	136, 93, 69, 41			0.0076	
2-Heptanone	14.11	1198	114, 58, 41			0.0042	
Hexanoic acid, methyl ester	14.26	1202	130, 99, 87, 74	0.0062	0.0106	0.0074	0.0052
D-Limonene	14.65	1212	136, 121, 93	0.1283	0.0016	0.0164	0.0376
1-Butanol, 2-methyl-	14.91	1219	88, 70, 57, 55	0.0079	0.0049	0.0083	0.0078
(2R,5R)-2-Methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran (50%)	15.14	1225	152, 137, 110, 67	0.0081	0.0009	0.0012	0.0012
1,3,8-p-Menthatriene	15.37	1231	134, 114, 91	0.0069	0.0015	0.0007	
(2R,5S)-2-Methyl-5-(prop-1-en-2-yl)-2-vinyltetrahydrofuran (73%)	16.39	1258	152, 137, 110, 67	0.0128	0.0014	0.0018	0.0019
β-Ocimene	16.69	1266	136, 93, 91	0.0076	0.0033	0.007	0.0008
Styrene	17.12	1278	104, 103, 78			0.0024	
o-Cymene	17.5	1288	134, 119, 91	0.0096	0.0037	0.0057	0.0039
Terpinolene	17.93	1299	136, 121, 93	0.0002		0.0084	
Heptanoic acid, methyl ester	18.06	1303	144, 113, 87, 74	0.0033	0.0038	0.0059	0.004
Octanal	18.19	1306	128, 110, 84, 57	0.0061	0.005	0.0097	0.0078
2-Propanone, 1-hydroxy (Aceto)	18.82	1324	74, 43, 31	0.0008		0.0014	
2-Heptanol	19.09	1331	116, 83, 55, 45	0.0023	0.0034	0.0026	0.0032
5-Hepten-2-one, 6-methyl (Sulcatone)	20.01	1356	126, 108, 69	0.0038		0.004	0.002
Benzene, 1-ethyl-3-methyl	20.11	1359	120, 105				0.001
1-Hexanol	20.36	1366	102, 84, 69, 56	0.0021	0.0048	0.002	0.002
Trans Rose oxide	20.51	1370	154, 139, 69	0.0006			
Limonene oxide	21.49	1397	152, 137, 108, 94	0.0039		0.0147	0.0068
Tetradecane	21.6	1400	198, 85, 71				0.0008
Octanoic acid, methyl ester	21.8	1405	158, 127, 87, 74	0.028	0.0247	0.0351	0.019
Nonanal	22.03	1412	142, 98, 82, 70	0.0354	0.0314	0.0345	0.0225
Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	23.72	1460	242, 155, 111, 94	0.018	0.0053	0.0154	0.0069
1-Octen-3-ol	23.82	1463	128, 85, 72, 57		0.0078		
Naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl- (α-Ionene)	24.02	1469	174, 159, 144	0.0018	0.001	0.003	0.0034
5-Hepten-2-ol, 6-methyl- (Sulcatol)	24.27	1476	128, 110, 95	0.0036			
Acetic acid	24.49	1482	60, 45, 43	0.0087	0.0073	0.0099	0.0049
Furfural	24.73	1489	96, 95, 67	0.0953	0.0495	0.2092	0.1192
Pyrazine, tetramethyl-	24.89	1493	136, 54, 42		0.0026		
1-Hexanol, 2-ethyl-	25.17	1501	112, 83, 57			0.025	0.0261
Nonanoic acid, methyl ester	25.39	1508	172, 87, 74	0.0437	0.0388	0.0472	0.0246
Decanal	25.71	1518	156, 112, 82, 57	0.0157	0.0165	0.016	0.0071
Ethanone, 1-(2-furanyl)- (Acetylfuran)	26.16	1531	110, 96, 95	0.0162	0.0088	0.0249	0.012
Dill ether (Anethofuran)	26.47	1540	152, 137, 109	0.0475	0.0051	0.0044	0.0054
Ethanone, 1-(1,4-dimethyl-3-cyclohexen-1-yl)-	26.65	1546	152, 137, 109, 67	0.0059		0.0023	
Benzaldehyde	26.91	1554	106, 105, 77	0.0062	0.004	0.0705	0.0309
2-Nonenal, (E)-	27.05	1558	140, 83, 70, 55		0.0007		0
β-Linalool	27.14	1560	154, 136, 121, 93	0.0961	0.0034	0.2074	0.0064
Lilac aldehyde A	27.28	1565	153, 111, 93	0.0294	0.0016	0.009	0.0024

Tables

Contin.

Volatile Compound	RT (min)	RI*	Fragment Ions *m/z*	Botanical origin of the honey			
				Citrus	Alfalfa	Marjoram	Black
1-Octanol	27.47	1570	84, 69, 56	0.0017	0.0019	0.0036	0.0028
Lilac aldehyde C	27.76	1579	153, 111, 93	0.0322	0.0017	0.0106	0.0028
Lilac aldehyde B	28.04	1587	153, 111, 93	0.0227	0.0012	0.0069	0.0018
3,5-Octadien-2-one	28.26	1594	124, 95, 81		0.0014		
2-Furancarboxaldehyde, 5-methyl-	28.49	1601	110, 109, 53	0.0047	0.0031	0.0125	0.0082
Decanoic acid, methyl ester	28.8	1611	186, 143, 74	0.0015	0.0095	0.0071	0.0022
Lilac aldehyde D	28.86	1613	153, 111, 93	0.0697	0.0011	0.0144	0.0041
2-Acetyl-5-methylfuran	29.06	1619	124, 109	0.0032	0.0003		0.0004
2,2'-Bifuran	29.18	1623	134, 105, 78		0.0005		
1,5,7-Octatrien-3-ol, 3,7-dimethyl- (Hotrienol)	29.25	1625	82, 71, 67	0.024	0.0024	0.0052	0.0058
3,9-Epoxy-1-p-menthene	29.32	1627	152, 138, 109, 93			0.0047	
1,4-Dimethyl-4-acetylcyclohexene	29.43	1631	152, 137, 109	0.0026			
2H-1-Benzopyran, 3,5,6,8a-tetrahydro-2,5,5,8a-tetramethyl-, trans- (Edulan I)	29.49	1632	192, 177, 133				0.0037
3-Cyclohexene-1-acetaldehyde, α,4-dimethyl-	29.88	1645	152, 95, 94, 79	0.1002	0.0064	0.0173	0.0071
3-Cyclohexene-1-acetaldehyde, α,4-dimethyl-	29.97	1647	152, 95, 94, 79	0.1174	0.0107	0.0202	0.01
Butanoic acid	30.16	1653	88, 73, 60				0.0017
Unknown (2)	30.33	1659	148, 138, 123				0.0009
Isomaltol	30.41	1661	126, 111	0.0021	0.0019	0.0022	
Nonanol	30.76	1672	144, 97, 83, 70	0.0092	0.0067	0.0014	0.003
Benzeneacetaldehyde	30.81	1674	120, 91, 65	0.0424	0.0121	0.1393	0.0387
2-Furanmethanol	30.96	1679	98, 97, 81	0.0082	0.0027	0.0061	0.0043
Benzoic acid, ethyl ester (Essence of niobe)	31.47	1695	150, 122, 105	0.0051	0.001	0.0129	0.0029
Estragole	31.53	1697	148, 147, 133				0.0007
Citral	31.9	1709	152, 84, 69	0.0019		0.0006	
3-Cyclohexene-1-acetaldehyde, α,4-dimethyl-	32.06	1715	152,95, 94, 79	0.0138	0.0062	0.0112	0.0068
α-Terpineol	32.17	1718	136, 121, 93	0.0203	0.015	0.0274	0.0054
Oxopholone	32.24	1721	152, 96, 68		0.0012		
Dodecanal (Lauraldehyde)	32.54	1731	140, 96, 82	0.0016	0.0012	0.0016	0.0006
Lilac alcohol C	33.06	1749	170, 155, 111, 93	0.008	0.0006	0.0041	0.0005
2-Hydroxycineole	33.18	1754	170, 126, 108				0.001
Lilac alcohol B	33.68	1771	170,155, 111, 93	0.0109	0.0009	0.0185	0.0011
1-Decanol	33.85	1777	158, 112, 97, 83	0.003	0.002	0.0023	0.0011
1, 1, 5-Trimethyl-1, 2-dihydronaphthalene	33.92	1779	172, 157, 142			0.0194	0.0055
Lilac alcohol A	34.72	1810	170,155, 111, 93	0.0094	0.0006	0.0064	0.0005
Methyl salicylate (Betula oil)	34.78	1813	152, 122, 92				0.0014
Dodecanoic acid, methyl ester	34.92	1820	214, 171, 87, 74	0.0349		0.0309	0.0075
Acetic acid, 2-phenylethyl ester	35.51	1848	105, 104, 91	0.0019			0.0043
Heptanoic acid, 2-ethyl-	35.55	1850	158,101, 88, 73		0.001	0.0039	
Lilac alcohol D	35.6	1852	170, 155, 111, 93	0.0036		0.0002	
β-Damascenone	35.65	1854	190, 121, 69	0.0005	0.0047		0.0091
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- (Nerol)	35.87	1865	154, 93, 69, 41			0.0025	
Hexanoic acid	36.02	1872	116, 87, 73, 60			0.0058	0.0052
α,β-Dihydropseudoionone	36.16	1879	194, 151, 136, 69	0.0064		0.0015	0.0014
Phenol, 2-methoxy- (Guajol)	36.43	1891	124, 109, 81			0.0019	0.0003
Benzyl alcohol	36.67	1905	108, 107, 79			0.0035	0.0077
cis-p-mentha-1(7),8-dien-2-ol	36.84	1917	134, 119, 109	0.0008			
p-Mentha-1(7),8(10)-dien-9-ol	37.18	1943	134, 119, 93	0.0037			
Phenylethyl Alcohol (Benzenethano or Rose oil)	37.28	1950	122, 92, 91	0.029	0.0051	0.0178	0.0065
3-Cyclohexene-1-ethanol, β,4-dimethyl- (p-Menth-1-en-9-ol)	37.51	1968	154, 121, 94	0.0107			
Hexanoic acid, 2-ethyl-	37.6	1974	144, 116, 88, 73	0.0059	0.0334	0.0055	0.0026
Heptanoic acid	37.66	1979	130, 87, 73, 60			0.0048	
2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)- (Jasmone)	37.75	1986	164, 149, 110	0.0014			
2,5-Furandicarboxaldehyde	38.19	2022	124, 123, 95		0.0018	0.0152	0.0078
Octanoic acid	38.88	2082	144, 115, 101, 73	0.011	0.0072	0.0166	0.0093
Decanoic acid, 3-hydroxy-, methyl ester	39.07	2098	202, 103, 71, 43	0.0053		0.006	0.0001
5-Hydroxymethylfurfural	40.18	2186	126, 109, 97	0.0839	0.1161	0.0124	0.1085
Nonanoic acid	40.22	2189	158, 129, 115, 73	0.0338	0.0198	0.0455	0.0191
n-Decanoic acid	41.75	2296	172, 143, 129, 73	0.0023	0.0013	0.0033	0.0014
Methyl anthranilate (Nevoli oil)	41.84	2302	151, 119, 92	0.0834			0.0027
8-Hydroxylinalool	42.26	2331	170, 137, 119, 71	0.0084		0.0005	

** Experimental athematic linear retention index.*

Table 2 Most significant compounds found in sugar syrup and their characterizing fragment ions

Compounds	RT	RI*	Conc. (µg.g^{-1})	Characteristic fragment ions, *m/z*	Selected ions *m/z*
Undecane	10.26	1095	0.0040	156, 99, 85, 71	99, 85, 71
5-methyl-2(3H)-Furanone	23.71	1460	0.0338	99, 98, 55, 43	98, 99
Furfural	24.73	1489	0.4964	97, 96, 95, 67	97, 96, 95
2-Furancarboxaldehyde, 5-methyl-	28.49	1601	0.0117	110, 109, 96, 81	110, 109, 96
2-methyl-Benzofuran	29.74	1640	0.0091	132, 131, 103	132, 131
Isomaltol	30.41	1661	0.0065	126, 111	126, 111
2-(2-furanylmethyl)-5-methyl-Furan	34.55	1850	0.0042	162, 161, 119, 91	162, 91
Hepta-2,4-dienoic acid, methyl ester	37.00	1929	0.0028	140, 111, 81	140
2,5-Furandicarboxaldehyde	38.19	2022	0.0043	124, 123, 95	124, 123
5-Hydroxymethylfurfural	40.18	2186	0.1739	126, 109, 97	126, 97
Nonanoic acid	40.22	2189	0.0143	158, 129, 115, 98, 73	73

** Experimental athematic linear retention index.*

Table 3 Statistical measures of PLS regression models developed on GC-MS and APCI-MS data for predicting the adulterant level in honey samples

Modelled Data	Calibration set					Validation set	
	LF	R_c^2	*RMSEC (%)*	R_{cv}^2	*RMSECV (%)*	R_p^2	*RMSEP (%)*
GC-MS	3	0.93	3.03	0.90	3.61	0.93	2.97
APCI-MS (Model I)	5	0.98	1.88	0.96	2.40	0.96	2.52
APCI-MS (Model II)	3	0.97	2.02	0.96	2.38	0.95	2.60