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Influence of beeswax adulteration with paraffin on the composition and quality of honey determined by physico-chemical analyses, ^{1}H NMR, FTIR-ATR and HS-SPME/GC-MS

***Abbreviated running title*:** Beeswax adulteration affects honey composition and quality

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Abstract

Analytical parameters were determined for the first time in honey produced in the honeycomb constructed on comb foundations adulterated with 90% of paraffin (PF-H) and compared to honey ripened in genuine beeswax (BWF-H) using physico-chemical and spectroscopic techniques (^{1}H NMR, FTIR-ATR, HS-SPME/GC-MS). Water content was significantly higher (SH) and glucose/water ratio significantly lower in PF-H samples. The contents of acetic and citric acid were marginally significantly higher (MSH) in PF-H samples. These findings suggest that adulterated beeswax affects composition of honey as the set of altered parameters indicate chemical changes leaning towards fermentative processes. Moderately changed headspace chemical profile of PF-H honey was determined depending on the floral source (pentanal, α-pinene and benzaldehyde were SH in BWF-H sunflower honey; butanal was MSH, and 2-phenylethanol was more abundant in BWF-H black locust honey). Higher percentage of nonanal, octane and β-damascenone were found in PF-H samples that could indicate more intensive oxidation.

Keywords: honey produced in paraffin-based ripening media; physico-chemical analyses; ^{1}H NMR; FTIR-ATR; HS-SPME/GC-MS; chemical alterations; fermentation susceptibility

1. Introduction

Honey production represents a complex biological process characterized by the conversion of nectar into honey involving both plant and animal input. This nectar-to-honey transformation process occurs in a honeycomb, a specific lipid based natural wax material produced by the honey bees (*Apis mellifera* L.) for brood development and food (nectar, pollen) storage. Honeycomb also represents a media where honey is being subjected to a ripening process. Honey ripening includes complex biochemical transformation process primarily involving evaporation of water content (below 20%), and conversion of sucrose into glucose and fructose which is mediated by both plant and honey bee enzymes. Nectary invertase hydrolyses sucrose to glucose and fructose during nectar secretion, while invertase secreted from the honey bee hypopharyngeal gland and salivary system (Winston, 1987) starts to act in the bee's honey sac immediately after collecting the nectar (Svečnjak, Prđun, Rogina, Bubalo, & Jerković, 2017), and continues in the honeycomb. The foodhandling bees evaporate nectar on their tongues before placing droplets in open honeycomb cells for further evaporation accelerated by fanning with their wings (Park, 1925). Once enough water has been evaporated, honey bees cap over the honey with wax cappings to allow further sugar conversion processes, i.e. hydrolysis of nectar sucrose to glucose and fructose until the concentration of these monosaccharides is about 82% - ripe honey (Park, 1925).

Thus, a honeycomb (beeswax) represents the first honey natural "packing material" (its temporary in-hive natural storage). However, despite its direct contact with honey (food), beeswax used for honey production (comb foundations) is nowadays not being subjected to any obligatory quality control prior to its placement on the market. Moreover, beeswax and its products used in the apicultural sector are classified as animal by-products (ABP) not intended for human consumption, and sub-categorized as category 3 material which includes ABPs that do not present a potential risk for the food chain as they must not contain residues

of other (foreign) substances and environmental contaminants (Reg. (EC) No. 1069/2009). However, beeswax is frequently marketed as "safe" category 3 even when it contains substances of questionable origin and chemical background (such as most commonly used adulterants, paraffin wax), due to the lack of obligatory legal regulations and clearly defined quality criteria. In this way, adulterated beeswax is regularly entering the beekeeping technology and honey production process *via* uncontrolled comb foundation production and trade.

As reported in numerous studies (Tulloch, 1973; Bogdanov, 2004; Serra Bonvehí, & Orantes Bermejo, 2012; Maia, Barros, & Nunes, 2013; Svečnjak, Baranović, Vinceković, Prđun, Bubalo, & Tlak Gajger, 2015; Waś, Szczęsna, & Rybak-Chmielewska, 2016), beeswax adulteration with paraffin wax represents a long present and growing problem worldwide. Paraffin is the most widely used beeswax adulterant due to its wide availability, low price, and physico-chemical properties (chemically inert, white or colourless and odourless substance) that altogether makes it "ideal" for beeswax adulteration (Svečnjak et al., 2015). Other adulterants, such as stearic acid, stearin, and tallow, are occurring sporadically (Svečnjak, Prđun, Bubalo, Matošević, & Car, 2016; Bogdanov, 2016; Reybroeck, & Van Nevel, 2018; Svečnjak, Prđun, Baranović, Damić, & Rogina, 2018). A recent study investigating the authenticity of 137 beeswax samples collected from the international market (15 European countries) revealed that >65% of analysed samples were adulterated with various share of paraffin (5 to 93%), while stearic acid was detected sporadically (Svečnjak et al., 2018).Given that contaminated beeswax frequently enters beekeeping technology and thus comes into contact with honey, this opens up a new aspect of beeswax adulteration issue - food safety. Moreover, in the case of comb honey trade (Council Directive 2001/110/EC), beeswax directly enters the food chain (although comb honey should be produced without previous insertion of comb foundation in the hive, this is often not the case in practice). The

negative legal and economic aspects of beeswax adulteration, as well as safety issues related to public and animal (honey bee) health, were recently brought to the attention of the European Commission (EC) by the EU Food Fraud Network; the material is currently under evaluation of the EC (EU Food Fraud network, 2017).

It can be assumed that changed chemical composition of beeswax caused by addition of foreign substances that are not an integral part of its composition (adulterants) could affect the composition and/or quality of honey ripened and stored in it. However, the influence of beeswax adulteration on the quality of honey has not yet been investigated. Therefore, the aim of this study was to investigate the chemical composition of honey ripened in adulterated beeswax (honeycomb constructed on comb foundations adulterated with 90% of paraffin) aiming to detect chemical alterations occurring in it compared to honey ripened in the natural environment (honeycomb built on comb foundations made of genuine beeswax). To investigate these changes, physico-chemical analyses, as well as ^{1}H NMR, FTIR-ATR and headspace solid-phase microextraction (HS-SPME/GC-MS) were applied.

2. Material and methods

2.1. Experimental design

A set of two types of comb foundations, one adulterated with paraffin, containing 90% of paraffin and 10% of beeswax (P), and the other made of genuine beeswax (BW) were inserted in 15 *A. mellifera* honey bee colonies placed in two super Langstroth-Root hives. The colonies were previously uniformed by strength according to the method described by Delaplane, Van der Steen, & Guzman (2013). Experimental comb foundations (placed into a comb frame with wires) were inserted in experimental beehives (paired as 1P:1BW set / hive), and placed in a honey super at lateral positions (BW at 3rd and P at 8th frame position) during the April - May period of the production season 2015.

The hives were situated on experimental apiaries located in different climatic-geographical regions of Croatia, Pannonian, Mountain, and Adriatic region (Fig. S1, Supplementary material), according to the following experiment setting:

1. ***Experimental apiary in the Western Pannonian Region (WPR)***

- 4 hives; dominant honeybee forage: black locust (*Robinia pseudoacacia* L.)

2. ***Experimental apiary in the Eastern Pannonian region (EPR)***

- 5 hives; dominant honeybee forage: sunflower (*Helianthus annuus* L.), multifloral

3. ***Experimental apiary in the Mountain region (MR)***

- 2 hives; dominant honeybee forage: honeydew

4. ***Experimental apiary in the Adriatic region (AR)***

- 4 hives; dominant honeybee forage: black locust (*Robinia pseudoacacia* L.), multifloral

Such experimental design was applied to ensure simultaneous production of honey in the same hive but in a different ripening media (honeycomb constructed on paraffin-based comb foundations - PF, and honeycomb constructed on uncontaminated comb foundation made of genuine beeswax - BWF), processed in the same conditions in terms of uniformity on biological (uniformed strength and vitality of the honeybee colonies, the same honey bee forage), technological (the same type of hive) and geo-climatic level (the same clime areas / geographical origin). In this way we reduced the experimental error and eliminated a possible influence of external factors on investigated honey compositional parameters.

Different geographical regions were chosen for honey production in order to obtain honey samples originating from different botanical sources (black locust, sunflower, honeydew, and multifloral honey), thus enabling a comprehensive and unbiased comparison of the obtained results given that numerous analytical values may vary significantly depending on the botanical and geographical origin of nectar source utilized for honey production.

Experimental honeycombs were left in the hives during the entire nectar flow period until honey stored in the combs was capped with wax cappings thus allowing usual nectar collection and honey ripening process in the hives.

2.2. Honey sampling

Experimental honeycombs with stored ripe honey were collected directly from the experimental hives during June - July period depending on the time required for honey ripening at different experimental locations. Both types of honey samples, honey ripened in the honeycomb built on paraffin-based (90%) comb foundations (PF-H), and honey ripened in the honeycomb constructed on comb foundations made of genuine beeswax (BWF-H), were collected simultaneously by manual honey extraction. Honeycombs were kneaded separately to obtain individual honey samples that were further filtered and stored in glass containers at room temperature in the dark until analyses.

2.3. Honey analysis using classical physico-chemical methods

Analysis of physico-chemical parameters, i.e. determination of water content, electrical conductivity and pH value of studied honey samples, was performed in accordance with Harmonized methods of the International Honey Commission (2009) and European legislation (Council Directive 2001/110EC). Water content was determined from the honey refractive index obtained by Mettler Toledo Refracto 30 PX refractometer (1320-1500 refractive index measuring range), electrical conductivity was measured by Mettler Toledo, LE703 conductivity meter (measurement range from 0.1 µS-199.9 mS/cm), while pH values was determined using Mettler Toledo MP 220 pH meter.

2.4. Honey analysis using spectroscopic techniques

2.4.1. Proton nuclear magnetic resonance spectrometry (^{1}H NMR)

600 µL of the sample (standard solution or pre-treated honey sample) were placed into a 5 mm outer diameter NMR tube, with 100 µL of a D_2O/H_2O solution containing 70% (v/v) D_2O and 10.0 g dm^{-3} of sodium 3-trimethylsilyl-3,3,2,2-tetradeuteriopropionate (TSP). The final concentrations were 10% D_2O and 1.43 g dm^{-3} of TSP. One-dimensional proton spectra were recorded on a Bruker Avance III HD 400 MHz/54 mm Ascend spectrometer equipped with a 5 mm PA BBI 1H/D-BB probehead with z-gradient and automated tuning and matching accessory. To obtain the spectra of the samples, 64 scans of 64 K data points were acquired at 300 K using a spectral width of 8012 Hz (20 ppm), acquisition time of 4.0 s, recycle delay of 2.0 s and a 90º flip angle, requiring about 7 min per sample. Water suppression was achieved by using the one-dimensional nuclear Overhauser effect spectroscopy pulse sequence, incorporating presaturation during the relaxation delay and mixing time (150 ms) (McKay, 2011) and the pre-saturation power used was the minimum needed to effect complete suppression of the water peak. The Free Induction Decay signals were processed before Fourier transformation using Bruker software, TOPSPIN 3.5; 32,768 data points were used, by applying an exponential line broadening of 0.3 Hz for sensitivity enhancement before Fourier transform and were accurately phased and baseline adjusted. Phase correction was performed manually for each spectrum, and the baseline correction was applied over the entire spectral range, using a simple polynomial curve fit included in TopSpin® software. The spectra were referenced to the TSP singlet peak at 0.0 ppm.

Linear regression analysis with integration ranges following del Campo et al. (2016) study were carried out with respect to each of 13 analytes considered. In this case ratio of analyte signal with signal for internal standard, TSP, achieved much better linear fit than using absolute areas for each analyte. 13 considered analytes in honey samples were glucose,

fructose, sucrose, acetic acid, citric acid, formic acid, lactic acid, succinic acid, alanine, phenylalanine, proline, tyrosine and hydroxymethylfurfural. Nine calibration standards were prepared using mentioned analytes with addition of histidine, maltose, ethanol, and KCl following amounts used by del Campo et al. (2016). For glucose, fructose and sucrose partial-least square regression (PLS) on the whole or specific spectral region were considered too. Optimal prediction models were selected using the best R^2 between true and predicted amounts in calibration samples, and then used for validation.

2.4.2. Fourier transform infrared spectroscopy (FTIR-ATR)

Infrared (IR) spectra of studied PF-H and BWF-H samples were recorded as obtained by Cary 660 Fourier transform mid-infrared spectrometer (Agilent Technologies) coupled with attenuated total reflectance (ATR) accessory (Golden Gate single-reflection diamond ATR), according to the method by Svečnjak et al. (2017) applied to acquire honey spectra.

2.4.3. Headspace solid-phase microextraction (HS-SPME) and chromatography - mass spectrometry (GC-MS)

Headspace solid-phase microextraction (HS-SPME)

The headspace solid-phase microextraction (HS-SPME) was performed using a manual SPME holder with divinylbenzene/carboxene/polydimethylsiloxane (DVB/CARB/PDMS) fiber (Supelco (Bellefonte, PA, USA). The honey/saturated NaCl water solution (5 mL, 1:1 (v/v)) was put in 15-mL glass vial and hermetically sealed with polytetrafluorethylene (PTFE)/silicone septa. The vial was placed in a water bath at 60 ^{o}C during equilibration (15 min) and headspace extraction (45 min) under constant stirring (1000 rpm) with a magnetic stirrer. After sampling, the SPME fiber was withdrawn into the needle, removed from the vial,

and inserted into the injector (250 ^{o}C) of the GC-MS for 6 min as previously reported (Jerković et al., 2016). The experiment was performed in duplicate for each sample.

Gas chromatography and mass spectrometry (GC-MS) analysis

The GC-MS analyses were performed with an Agilent Technologies (Palo Alto, CA, USA) gas chromatograph model 7820A containing a mass selective detector (MSD) model 5977E (Agilent Technologies) and a HP-5MS capillary column ((5% phenyl-methylpolysiloxane, Agilent J and W, Santa Clara, CA, USA). The oven temperature was programmed isothermal at 70 ^{o}C for 2 min, increasing from 70–200 ^{o}C at 3 ^{o}C·min^{-1}, and held isothermally at 200 ^{o}C for 15 min. The carrier gas was He (1.0 mL·min^{-1}) and the total run time was 65 min. The MSD (EI mode) was operated at 70 eV, and the mass range was 30-300 amu, as previously reported (Jerković et al., 2016). The identification of the headspace compounds was based on the comparison of their retention indices (RI), determined relative to the retention times of C9-C25 homologous series of n-alkanes with those reported in the literature and on the comparison of their mass spectra with available authentic compounds or with the mass spectra listed in Wiley 9 (Wiley, New York, NY, USA) and NIST 14 (D-Gaithersburg) mass spectral libraries. The percentage composition of the samples was computed from the GC peak areas using the normalization method (without correction factors). The individual component percentages in Table 3 were calculated from duplicate GC-MS analyses.

2.4.4. Statistical analysis

In order to assess the influence of different ripening media (paraffin-based honeycomb vs. genuine beeswax) on honey composition and determine the statistical significance of observed differences, the mean values of analysed PF-H / BWF-H paired set of physico-chemical data, SPME/GC-MS results, and ^{1}H NMR measurement data were compared using Paired sample t-test ($p<0.05$). Furthermore, data were analysed by means of descriptive

statistics and classical one-way analysis of variance (ANOVA) to assess the differences in indicative physico-chemical and ^{1}H NMR measurement data between PF-H and BWF-H samples in relation to different geographical (four locations) and botanical origin (four honey tapes) by statistical software package Statistica - StatSoft v.7.

3. Results and discussion

In total 30 honey samples collected from the experimental honeycombs, paired as 15 PF-H vs. 15 BWF-H, were categorized by their botanical origin based on reference parameters: electrical conductivity values, honey type confirmation by qualitative melissopalynological analysis (Von der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004), and sensory analysis (Piana, Persano Oddo, Bentabol, Bruneau, Bogdanov, & Guyot Declerck, 2004), as follows (distribution of honey types according to production location):

1. WPR: black locust (*Robinia pseudoacacia* L.) honey; n=8
2. EPR: sunflower (*Helianthus annuus* L.) + multifloral honey; n=4+6=10
3. MR: honeydew honey; n=4
4. AR: black locust (*Robinia pseudoacacia* L.) + multifloral honey; n=4+4=8

3.1. Determination of physico-chemical parameters

The results revealed that the values of water content differed between the honey samples ripened in different media (paraffin-based vs. genuine beeswax) indicating biochemical alterations in honey related to changed maturation environment (beeswax adulterated with paraffin). As presented in Table 1 showing descriptive statistics and effects of one-tail Paired sample t-test comparing the mean values of analysed PF-H vs. BWF-H paired set of measurement data, the results revealed that the mean water content value was significantly higher ($p=0.024$) in honey samples ripened in honeycomb built on paraffin-based comb

foundations (PF-H) compared to the honey samples ripened in honeycomb built on genuine beeswax (BWF-H). The water content values were higher in 13 out of 15 PF-H honey samples (86.7%) compared to paired BWF-H samples from the same hive. The increased value difference per individual PF-H / BWF-H set of samples ranged from 0.1 to 2.7%, with an average increase difference of 0.41% in PF-H honey samples (Table S1, Supplementary material). Opposite results (slightly higher values determined for BWF-H samples) were observed in case of two paired set of sunflower honey samples from the Eastern Pannonian region, as presented by means of descriptive statistics of analysed samples according to botanical and geographical origin (Table S1, Supplementary material), and by one-way ANOVA, as presented in Fig. 1A/B, respectively. These reverse effects could be explained by the weather conditions (high daily temperatures >27°C; relative humidity = 63%) reported in EPR during the experiment period which might led to slower evaporation of water regardless of ripening media. Classical one-way ANOVA showed no statistically significant differences in water content value between PF-H and BWF-H samples in relation to the botanical origin (Fig. 1A) or production location / geographical origin (Fig. 1B).

Water content in honey is the most important quality parameter for the shelf life of honey because it determines the ability of honey to resist fermentation and remains stable. Optimal water evaporation in a beehive is enabled by both natural storage media made of beeswax in which honey is being kept during ripening, and the work of honey bees that fan the nectar stored in the honeycomb with their wings to evaporate the excess water (Hepburn, Pirk, & Duangphakdee, 2014; Nicolson, 2009; Park, 1925).

In relation to this, higher water content observed in most of studied PF-H samples indicate slower evaporation of water in honey samples ripened in unnatural paraffin-based media compared to genuine beeswax. This might be related to the simple chemical composition of paraffin (alkanes of different chain lengths), as opposed to a complex chemical structure of

beeswax (lipid-based wax consisted dominantly of fatty acid esters) which naturally ensures normal water transfer process (water evaporation) during the honey ripening. During the comb wax construction, lipolytic enzymes are added to the beeswax by the honey bees to ensure chemical changes necessary to form combs with higher monoacylglycerol content than the diacylglyceride-richer wax scales (Davidson & Hepburn, 1986). In this way, the degree of saturated bonds in constructed comb wax is increased which contribute to specific consistency and firmness of constructed honeycombs. However, the activity of these enzymes requires certain portion of an aqueous medium in beeswax. Although beeswax is primarily hydrophobic, the esters, carboxyl groups of free fatty acids and the hydroxyl groups of free alcohols in beeswax are hydrophilic, which allows mentioned processes to occur (Hepburn et al, 2014). Thus, the background mechanisms causing water increasing effects in honey ripened in paraffin-based environment could be explained by the hydrophobic nature of paraffin wax that might block normal moisture transfer during the honey maturation. Regardless of the background, this effect is unquestionably negative given that the higher water content in honey greatly contributes to fermentation, as the most important biological degradation of honey.

Negative effects regarding comb construction were also observed in case of honeycomb construction on PF; deformed honeycombs and the loss of a normal hexagonal structure of the honeycomb cells (Fig. S2, Supplementary material). In addition, a decreased honey yield in paraffin-based honeycombs was also observed as a side-line study observation. However, these observations were not supported by metric data in this study, but should be further investigated by appropriate in field studies.

Other physico-chemical parameters, electrical conductivity and pH value, showed no statistically significant differences between PF-H and BWF-H samples in relation to ripening media (Table 1). Moreover, the average values of these parameters were almost equal for both

PF-H and BWF-H samples. Descriptive statistics revealed that electrical conductivity values of analysed honey samples (Table S2, Supplementary material) were ranged within the range values determined for particular honey types compared to data from the literature (Persano Oddo & Piro, 2004). Besides normal variations related to different botanical origin, i.e. honey types (Table S2, Supplementary material), the results of Paired sample t-test showed no statistically significant differences ($p=0.451$) in relation to different ripening media (Table 1). Moreover, the mean values of electrical conductivity were equal (0.42 mS/cm) for both PF-H and BWF-H sample sets. The fact that electrical conductivity of honey is primarily related to the mineral composition of the nectar source that is not being subjected to subsequent chemical transformations in the honeycomb, explains the results obtained.

Furthermore, it was observed that different ripening media did not affect pH values of honey samples; the results of Paired sample t-test revealed no statistical significance when comparing mean pH values of paired PF-H and BWF-H samples ($p=0.179$). The results revealed similar degree of congruence within pH range and mean values among PF-H and BWF-H honey samples (Table 1), as well as insignificant variations common for different types of honey (Table S3, Supplementary material).

3.2. Honey analysis by ^{1}H NMR

Table S4 (Supplementary material) displays results of each of considered ^{1}H NMR analytes ($n=13$) in 30 honey samples according to ripening origin (PF vs. BWF), complemented with additional calculations of sum of glucose and fructose, and glucose / water ratio, while statistical parameters determined for ^{1}H NMR measurement data are summarized in Table 2, presenting descriptive statistics and Paired sample t-test effects. The statistical analysis of macro (glucose, fructose) and micro constituents (sucrose, amino acids, organic acids, HMF) of honey by Paired sample t-test showed several statistically significant differences indicating

honey quality issues arising from the influence of paraffin-based ripening media. Following the results of increased water content in PF-H samples, the results of Paired sample t-test revealed that the glucose / water ratio was significantly lower ($p=0.047$) in honey samples ripened in the honeycomb built on PF (Table 2). Both water-relating observations indicate negative effects on the composition and quality of honey stored in the honeycomb contaminated with paraffin by altering the ripening process in terms of slower evaporation of water.

An exception arising from higher glucose / water ratio was observed in sunflower BWF-H samples from EPR, as the higher water content was determined in those samples. Along with *Brasicca* and *Taraxacum* honey types, sunflower honey is characterized by high amount of glucose and total monosaccharides, as well as high value of glucose / water ratio (Persano Oddo & Piro, 2004) so these reverse effects could be explained by mentioned compositional properties of sunflower unifloral and sunflower-dominated multifloral honey (in addition to previously mentioned weather conditions reported in EPR during the experiment period).

The results have also revealed that the contents of acetic and citric acid were marginally significantly higher ($p=0.098$ and $p=0.084$, respectively) in PF-H samples (Table 2). Although present in small quantities, less than 0.5% respectively, organic acids in honey are important micro-constituents as they contribute to honey's physico-chemical and organoleptic properties, but may also be indicators of incipient fermentation process in honey (Mato, Huidobro, Simal-Lozano, & Sancho, 2003). The predominant acid found in honey is gluconic acid whose presence in all honey types originates from the activity of glucose oxidase provided by the bees during ripening (Mato et al., 2003; Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014). However, given that gluconic acid was not investigated in this study, citric acid was the most dominant organic acid in analysed samples which is in accordance with the results on citric acid fraction as the largest acid fraction in honey after

predominant gluconic acid, as reported and reviewed in numerous studies (Stinson, Subers, Petty, & White, 1960; Mato et al., 2003; Karabagias et al., 2014; Missio da Silva, Gauche, Valdemiro Gonzaga, Oliveira Costa, & Fett, 2016). Even though acetic acid is generally found in most honey types, an excessive acetic acid concentration indicate fermentation but there are still no studies of normal and fermentation levels of acetic acid in honey (Mato et al., 2003).

Along with significantly higher water content ($p=0.024$) and significantly lower glucose/ water ratio ($p=0.047$), acetic and citric acid increase determined in PF-H samples, strongly suggest negative chemical alterations related to changed (paraffin-based) ripening media, primarily in terms of honey's susceptibility to fermentation. No statistically significant differences between PF-H and BWF-H samples were found among other honey constituents analysed by ^{1}H NMR, as showed in Table 2.

The results of one-way ANOVA revealed no statistically significant difference in acetic and citric acid content between PF-H and BWF-H samples in relation to the botanical origin / honey type (Fig. 2A-B), and geographical origin / production location (Fig. 2C-D).

3.3. FTIR-ATR results and spectral data analysis

Given that honey can be described as a saturated aqueous solution of monosaccharides (glucose and fructose), the characteristic IR spectrum of honey is dominated by spectral features due to H_2O, glucose and fructose (Max & Chapados; 2007; Wang, Kliks, Jun, Jackson, & Li, 2010; Svečnjak et al., 2017). The major differences between PF-H and BWF-H honey spectra were observed in the fingerprint region between 1500 and 750 cm^{-1} (Fig. 3A and 3B, respectively); it was observed that the spectral variations occurring in this region (especially in sugar-based spectral envelope between 1175 and 900 cm^{-1} populated by medium and strong analyte signals belonging primarily to glucose and fructose), were more

uniform in BWF-H samples compared to PF-H samples (Fig. 3C and 3D). This indicates a more stable concentration of fructose and glucose in the natural (beeswax) ripening media. These findings are in compliance with results on sugar content obtained by ^{1}H NMR (Table S4, Supplementary material) and might be associated to inadequate enzyme activity (invertase and/or glucose oxidase) in unnatural ripening media, which should be further investigated.

In order to investigate average spectral variations between honey samples ripened in different ripening media, averaged spectrum of 15 PF-H vs. 15 BWF-H honey samples were compared, The results have revealed that the most of integrated absorbance intensities of the averaged PF-H and BWF-H honey spectra did not differ (Fig. 3E); only the band with absorption maximum at 1643 cm^{-1} belonging to H-O-H deformation vibration showed higher absorbance intensity in PF-H averaged honey spectrum (Fig. 3F). These spectral observations confirmed the results obtained by classical physico-chemical determination of water content showing the higher average amount of water in PF-H samples.

3.4. SPME/GC-MS identification of the headspace volatile organic compounds

The headspace volatile organic compounds (HS-VOCs) from PW-H and BWF-H honey samples were investigated by headspace solid-phase microextraction (HS-SPME) followed by GC-MS analyses. SPME/GC-MS identification of HS-VOCs has been useful tool for the characterisation and chemical profiling of different honey types (Wolski, Tambor, Rybak-Chmielewska, & Kędzia, 2006; Kaškonienė & Venskutonis, 2010; Manyi-Loh, Ndip, & Clarke, 2011; Jerković & Kuś, 2014; Jerković & Kuś, 2017) and it was therefore applied for the current research.

As presented in Table 3, the obtained HS-VOC profiles from the honey samples originated from different geographical regions depend on the available nectar flow sources as well on the ripening media (honeycomb built on PF/BWF). The differences/similarities among PF-H and

BEF-H samples were separately discussed for the samples from the same region regarding major compounds percentage and noted differences among the compounds abundance (after the statistical analysis by Paired sample t-test). Four samples from Mountain region were not investigated for the presence of HS-VOCs (due to insufficient amount of the samples for comparison and statistical analysis).

The samples from EPR region (with major sunflower nectar flow) from both types of ripening media predominated with (mean PF-H; mean BWF-H, Table 3): hotrienol (13.79%; 7.95%), and furfural (18.49%; 22.38%). Besides hotrienol, other linalool derivatives were also abundant such as *cis*- and *trans*-linalool oxides (Table 3). Furfural ranged from 10.23 to 27.37% in PF-H samples, and in BWF-H samples from 15.81 to 27.01%. Benzene derivative benzaldehyde ranged from 1.98 to 4.27% in PF-H samples, and in BWF-H samples from 3.76 to 12.12%, while monoterpene α-pinene was represented with 1.00 to 2.40% in PF-H samples, and from 2,48 to 3.78% in BWF-H samples. An array of other compounds was also found (Table 3). In comparison to available reports on unifloral sunflower honey, α-pinene or 3-methylbutan-2-ol were found by purge-and-trap technique (TenaxTM TA trap; Radovic, Careri, Mangia, Musci, Gerboles, & Anklam, 2001) while octanal, phenylacetaldehyde, octan-1-ol, 2-methoxyphenol, nonanal and 2-H-1-benzopyran-2-one were found by HS-SPME/GC-MS (Baroni, Nores, Díaz, Chiabrando, Fassano, Costa, & Wunderlin, 2006). The statistical analysis revealed differences between PF-H and BWF-H samples; the percentages of pentanal, α-pinene and benzaldehyde were significantly higher ($p=0.037$; $p=0.044$; $p=0.033$, respectively) in the honey samples originating from BWF ripening media. Those compounds are important for the honey characterisation since e.g. pentanal was reported as a chemical marker for buckwheat honey, α-pinene can be associated with plants as typical plant compound and it was reported as the chemical marker of sunflower honey, and benzaldehyde is typical honey constituent (Kaškonienė & Venskutonis, 2010; Manyi-Loh et al., 2011). In

addition, hexanal (p=0.099) and furfural (p=0.097) were marginally significantly higher in BWF-H samples. Hexanal was found as chemical marker of black locust honey, while furfural was found to be important compound of buckwheat honey (Manyi-Loh et al., 2001; Kaškonienė & Venskutonis, 2010).

The dominant compounds of the samples from WPR region (with major black locust nectar flow) were (av. PF-H; av. BWF-H, Table 3): hotrienol (17.65%; 14.20%), *cis*-linalool oxide (24.26%; 28.24%), and furfural (13.24%; 7.85%). Other abundant monoterpenes were *trans*-linalool oxide (5.87%; 6.51%), and linalool (3.40%; 3.06%). Identified minor constituents are listed in Table 3. *cis*-Linalool oxide and heptanal were found by purge-and-trap (TenaxTM TA trap) as characteristic compounds of black locust honey (Radovic et al., 2001), and hexanal by HS-SPME/GC-MS/olfactometry (Wardencki, Chmiel, Dymerski, Biernacka, & Plutowska, 2009). The statistical analysis showed that butanal percentage was marginally significantly higher (p=0.097), while benzaldehyde percentage (p=0.120) was close to marginally significantly higher values in BWF-H samples. Contrary, octane (p=0.095) and β-damascenone (p=0.093) were marginally significantly higher in PF-H samples. Octane as alkane could partially derive from paraffin-based comb foundations, while β-damascenone is norisoprenoid formed by degradation of carotenoids and oxidation that could also be more promoted in paraffin-based ripening media.

The major HS-VOCs of the samples from Adriatic region (with major black locust nectar flow) were (mean PF-H; mean BWF-H): butanal (12.18%; 12.87%), furfural (15.32%; 14.86%), benzaldehyde (20.8%; 19.87%), nonanal (8.88%; 3.70%), and *cis*-linalool oxide (7.39%; 3.03%). Hotrienol was also abundant (1.32; 2.99) followed by minor constituents (Table 3). The results of the statistical analysis revealing differences between PF-H and BWF-H samples are further presented: nonanal percentage was significantly higher (p=0.034) in the samples from paraffin-based honeycombs that could generally indicate more pronounced

oxidation process in PF-H honey samples. *cis*-Linalool oxide percentage was marginally significantly higher (p=0.072) in PF-H samples, while close to the marginally significantly higher values were the percentages of *trans*-linalool oxide (p=0.157), and linalool (p=0.143) in PF-H samples, as well as 2-phenylethanol (p=0.121) and decanal (p=0.107) percentages in BWF-H samples. Therefore, linalool derivatives seems to be more pronounced in PF-H samples indicating more intensive linalool transformation in honey samples from PF ripening media. However typical honey compound 2-phenylethanol (Wolski et al., 2006) was in general more abundant in BWF-H samples.

Considering crucial findings obtained in this study, we propose the initial combination of water content determination and spectroscopic profiling (using SPME/GC-MS and ^{1}H NMR) to be used for further studies aiming to discriminate PF-H (or honey ripened in beeswax contaminated with other type of adulterant) from BW-H samples.

4. Conclusions

The results obtained in this study represent the first record on the influence of beeswax adulteration on the composition and quality of honey based on the experiment employing one of the most commonly used beeswax adulterant - paraffin wax.

The results revealed that water content was significantly higher (p=0.024) and glucose / water ratio significantly lower (p=0.047) in honey samples ripened in paraffin-based ripening media, while the content of acetic and citric acid was marginally significantly higher (p=0.098 and p=0.084, respectively) in PF-H samples. These findings indicate that beeswax adulteration with paraffin affects normal nectar-to-honey transformation pathway and strongly suggest that unnatural paraffin-based honey ripening media is causing negative chemical alterations in honey leaning towards fermentative processes, i.e. honey's susceptibility to fermentation, regardless of honey's botanical and/or geographical origin.

In addition, it was found that PF ripening media moderately changed the HS-VOCs chemical profile of PF-H honey in comparison to BWF-H honey from the same hive and location. The percentages of pentanal, α-pinene and benzaldehyde were significantly higher ($p=0.037$; $p=0.044$; $p=0.033$, respectively) in BWF-H sunflower - based honey samples compared to PF-H samples from the same hive / location, butanal percentage was marginally significantly higher ($p=0.097$) in BWF-H black locust honey samples from WPR, while typical honey compound, 2-phenylethanol, was in general more abundant in black locust BWF-H samples from AR. Mentioned volatile compounds were previously reported as honey chemical markers. Contrary, a higher percentage of several compounds were determined in honey samples ripened in paraffin-based media: nonanal, octane and β-damascenone, namely. In addition, *cis*-linalool oxide was higher in all PF-H samples with an exception of black locust PF-H from WPR. A higher amount of these compounds could be chemically linked to paraffin presence followed by more intensive oxidation processes in honey ripened in paraffin-based media.

It can be generally concluded that important honey quality parameters, primarily water and acetic acid content as fermentation indicators, as well as nonanal, octane and β-damascenone, as indicators of oxidation processes, were increased in PF-H compared to BWF-H samples.

Along with negative effects of beeswax adulteration (paraffin-based ripening media) on honey quality parameters demonstrated in this study, a concern in terms of its impact in the context of food safety should also be accentuated here given that the chemical background of various paraffins and/or other substances used as beeswax adulterants is unknown, which represents a threat to public health as honey of questionable quality is continuously entering the food chain. This implies an urgent need for risk assessment, the implementation of mandatory regulations defining the beeswax quality criteria, as well as routine control of beeswax (comb foundations) used for honey production prior to its placement on the market.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure captions:

Fig. 1. Effects of one-way ANOVA showing differences in the mean water content (%)between honey samples ripened in the paraffin-based media (PF-H; n=15) and in genuine beeswax media (BWF-H; n=15) in relation to botanical origin (A) and production location / geographical origin (B)

Fig. 2. Effects of one-way ANOVA showing differences in the mean acetic and citric acid content (%) between honey samples ripened in the paraffin-based media (PF-H; n=15) and in genuine beeswax media (BWF-H; n=15) in relation to botanical origin (acetic acid - A; citric acid - B) and production location / geographical origin (acetic acid - C; citric acid - D)

Fig. 3. FTIR-ATR spectral variations (fingerprint region: 1500 - 700 cm^{-1}) between PF-H honey samples ripened in paraffin-based honeycomb (n=15; A) and BWF-H samples ripened in genuine beeswax-based honeycomb (n=15; B) in the same hive; sugar-based spectral envelope (1175-900 cm^{-1}) presenting variations in PF-H samples (C) compared to variations within BWF-H samples (D); averaged FTIR ATR spectrum (whole spectral region: 4000-400 cm^{-1}) of 15 PF-H and 15 BWF-H honey samples (E); averaged FTIR ATR spectrum (H_2O deformation vibration with absorption maximum at 1643 cm^{-1}) of 15 PF-H and 15 BWF-H honey samples (F)

*δ = *in-plane* bending vibration (deformation)

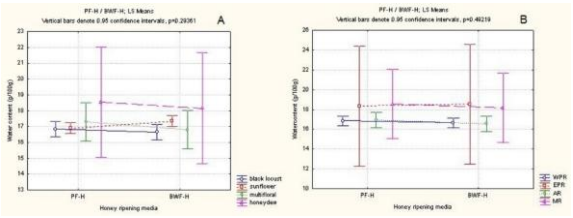
ν = *in-plane* stretching vibration

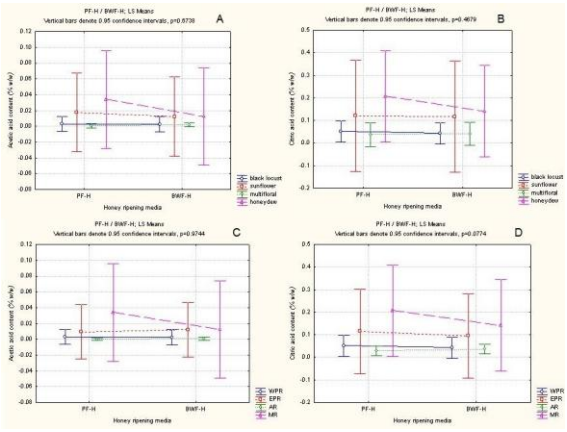
ω = *out-of-plane* bending vibration (wagging)

Glu = glucose

Fru = fructose

Suc = sucrose





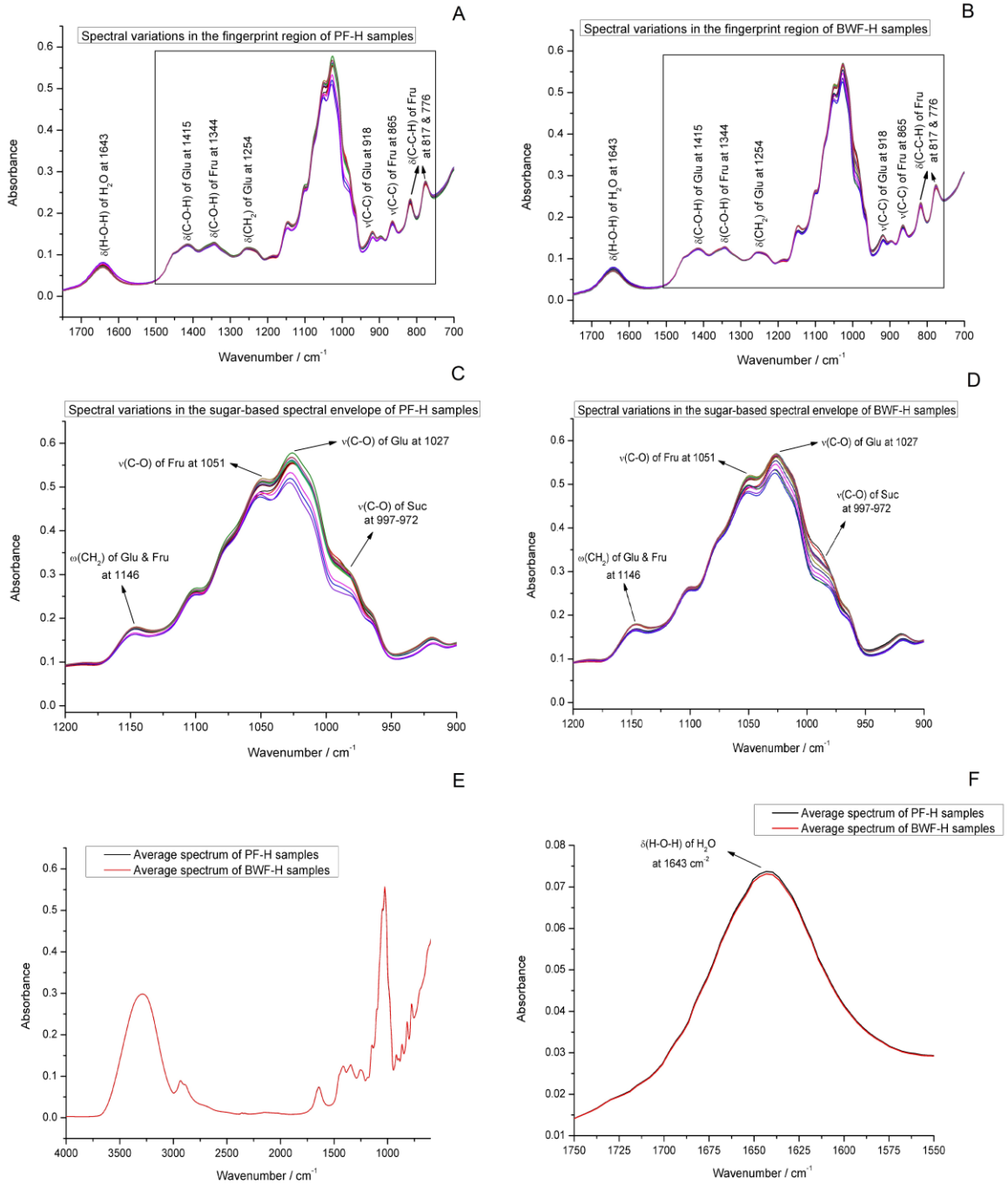


Table 1. Descriptive statistics and Paired sample t-test effect showing statistical significance of differences between determined physico-chemical values of paired set of PF-H / BWF-H honey samples produced in paraffin based beeswax (PF-H, n=15) and genuine beeswax (BWF-H, n=15)

Paired sample t-test parameters (at 0.05 significance level)		**Water content (g/100g)**	**Electrical conductivity (mS/cm)**	**pH value**
Parameter	Ripening media			
$\bar{x}$	PF	17.76	0.42	4.10
	BWF	17,35	0.42	4.08
Minimum	PF	16.30	0.18	3.71
	BWF	16,20	0.11	3.72
Maximum	PF	20.00	0.97	4.77
	BWF	19,80	0.89	4.72
SD	PF	1.32	0.24	0.32
	BWF	1,10	0.25	0.33
p (T<=t) one-tail		***0.0245***	0.4513	0.1794

Table 2. Descriptive statistics and Paired sample t-test effect showing statistical significance of differences between honey produced in paraffin based honeycomb (PF-H, n=15) and in genuine beeswax honeycomb (BWF-H, n=15) determined for paired PF-H/BWF-H ^{1}HNMR measurement data (amounts are presented in % *w*/*w*)

Par.	Ripen. media	Fructose[a] (%)	Glucose[a] (%)	Sucrose[a] (%)	Glucose+ Fructose (%)	Glucose / water ratio	Phenylalanine (%)	Proline (%)	Tyrosine (%)	Alanine (%)	Acetic acid (%)	Citric acid (%)	Formic acid (%)	Lactic acid (%)	Succinic acid (%)	HMF (%)
$\overline{x}$	PF	40.4124	40.57567	1.5749	80.98804	2.2870	0.0135	0.0840	0.0046	0.0071	0.0101	0.0953	0.0070	0.0171	0.0134	0.0056
	BWF	39.9159	41.3820	1.8347	81.2980	2.3881	0.0137	0.0818	0.0050	0.0077	0.0068	0.0847	0.0077	0.0192	0.0123	0.0056
Min.	PF	33.3481	33.442	0	79.1392	1.9443	0	0.0261	0	0	0	0.0165	0.0032	0.0002	0.0018	0.0016
	BWF	33.027	34.5507	0	78.6349	2.0324	0	0.0225	0	0	0	0.0160	0.0036	0.0006	0.0011	0.0018
Max.	PF	46.1835	46.9661	3.3588	82.8119	2.71598	0.0929	0.2036	0.0231	0.0315	0.0530	0.2406	0.0155	0.0570	0.0574	0.0142
	BWF	45.599	47.3403	5.061	83.7961	2.7364	0.1012	0.1708	0.0270	0.0323	0.0227	0.1984	0.0261	0.0800	0.0584	0.0129
SD	PF	4.1907	4.29410	1.04594	1.1157	0.2092	0.0244	0.0554	0.0059	0.0105	0.0148	0.0766	0.0037	0.0198	0.0173	0.0037
	BWF	4.2383	3.8958	1.6234	1.3656	0.2042	0.0255	0.0509	0.0067	0.0115	0.0084	0.0650	0.0056	0.0264	0.0144	0.0035
p (T<=t) one-tail		0.2137	0.1475	0.2277	0.2186	***0.0465***	0.4260	0.3051	0.1932	0.2223	***0.0982***	***0.0841***	0.3147	0.1989	0.2841	0.4785

Table 3. Descriptive statistics and Paired sample t-test effect showing statistical significance of differences between determined headspace volatile organic compounds of honey samples produced in paraffin based beeswax (PF-H, n=15) and genuine beeswax (BWF-H, n=15) according to production location and honey type (EPR= Eastern Pannonian region, WPR= Western Pannonian Region; AR= Adriatic region)

No	Compound (RI) / Paired sample t-test effect	Area percentages (%)																							
		PF-H (EPR, sunflower)				BWF-H (EPR, sunflower)				PF-H (WPR, black locust)				BWF-H (WPR, black locust)				PF-H (AR, black locust, multifloral)				BWF-H (AR, black locust, multifloral)			
		Min	Max	$\overline{X}$	SD	Min	Max	$\overline{X}$	SD	Min	Max	$\overline{X}$	SD	Min	Max	$\overline{X}$	SD	Min	Max	$\overline{X}$	SD	Min	Max	$\overline{X}$	SD
1.	Ethanol (< 900)	0.00	6.01	2.46	2.97	0.00	3.23	1.36	1.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	0.1573								-								-							
2.	Acetaldehyde (< 900)	-	-	-	-	-	-	-	-	0.00	12.35	4.12	7.13	-	-	-	-	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	-								0.2113								-							
3.	Butanal (< 900)	-	-	-	-	-	-	-	-	-	-	-	-	0.00	6.12	3.53	3.16	9.98	14.66	12.18	2.35	0.00	20.82	12.87	11.25
	p (T<=t) one-tail	-								***0.0967***								0.4617							
4.	Methylthio-methane (< 900)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.31	10.98	7.37	3.37	0.00	8.47	5.23	4.57
	p (T<=t) one-tail	0.3946								-								0.1299							
5.	Hexane (< 900)	0.00	9.73	4.45	5.18	0.00	7.27	1.82	3.64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	0.1742								-								-							
6.	3-Methylbutanal** (< 900)	0.91	2.12	1.56	0.51	0.00	2.82	1.54	1.17	-	-	-	-	-	-	-	-	0.00	2.00	0.67	1.15	-	-	-	-
	p (T<=t) one-tail	0.4749								-								0.2113							
7.	Pentanal (< 900)	0.00	3.12	2.02	1.40	3.20	6.44	4.79	1.32	0.00	2.09	0.70	1.21	0.00	1.81	1.00	0.92	0.00	3.66	1.51	1.91	0.00	8.60	3.81	4.38
	p (T<=t) one-tail	***0.0374***								0.2853								0.2441							
8.	2-Methylbutanal** (< 900)	0.00	2.54	1.18	1.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	0.0919								-								-							
9.	Octane (< 900)	-	-	-	-	-	-	-	-	0.00	1.81	1.07	0.95	-	-	-	-	0.00	3.99	1.33	2.31	0.00	5.93	3.21	2.99
	p (T<=t) one-tail	-								0.0951								0.1102							
10.	Hexanal (< 900)	1.13	2.54	1.70	0.69	1.48	3.58	2.28	0.99	0.00	2.09	0.70	1.21	0.00	1.81	1.00	0.92	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	0.0987								0.2853								-							
11.	2-Furan-carboxaldehyde (Furfural) (< 900)	10.23	27.37	18.49	8.67	15.81	27.01	22.38	5.30	1.39	25.50	13.24	12.06	2.77	15.36	7.85	6.64	7.82	23.66	15.32	7.95	9.72	19.76	14.86	5.02
	p (T<=t) one-tail	0.0970								0.2990								0.4780							
12.	α-Pinene (942)	1.00	2.40	1.87	0.63	2.48	3.78	2.90	0.59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	***0.0443***								-								-							
13.	Benzaldehyde (965)	1.98	4.27	3.31	1.04	3.76	12.12	7.30	3.53	3.17	17.53	10.15	7.19	16.16	27.98	22.78	6.03	17.25	24.29	20.08	3.72	10.16	29.83	19.87	9.84
	p (T<=t) one-tail	***0.0327***								0.1195								0.4899							

14.	Benzyl alcohol (1043)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00	1.51	0.50	0.87
	p (T<=t) one-tail	-								-								0.2113							
15.	Phenylacetaldehyde (1048)	0.00	1.07	0.47	0.46	0.00	1.11	0.62	0.57	0.00	1.58	0.99	0.86	0.00	4.64	1.63	2.61	3.12	8.63	5.41	2.87	0.00	8.75	3.64	4.56
	p (T<=t) one-tail	0.2919								0.3907								0.1665							
16.	*cis*-Linalool oxide (furan type) (1076)	4.10	13.19	9.56	3.99	7.12	10.73	8.93	1.48	18.84	32.25	24.26	7.06	22.88	30.99	28.24	4.65	5.67	10.52	7.39	2.71	0.00	5.03	3.03	2.67
	P (T<=t) one-tail	0.3489								0.2190								0.0722							
17.	*trans*-Linalool oxide (furan type) (1091)	1.27	4.99	3.12	1.71	2.23	4.40	3.31	1.15	4.88	7.67	5.87	1.56	5.60	7.12	6.51	0.80	0.00	2.98	1.62	1.51	0.00	1.51	0.89	0.79
	p (T<=t) one-tail	0.3161								0.2637								0.1570							
18.	Linalool (1101)	0.00	1.14	0.28	0.57	-	-	-	-	2.37	4.34	3.40	0.98	2.36	4.16	3.06	0.96	4.25	5.16	4.75	0.46	0.00	4.36	2.51	2.25
	p (T<=t) one-tail	0.1955								0.2482								0.1425							
19.	Nonanal (1107)	-	-	-	-	-	-	-	-	0.00	3.79	1.26	2.19	-	-	-	-	7.59	11.05	8.88	1.89	0.00	7.04	3.70	3.53
	p (T<=t) one-tail	0.2113																***0.0337***							
20.	Hotrienol (1112)	7.34	25.94	13.79	8.30	5.78	12.17	7.95	2.87	10.92	26.49	17.65	8.00	11.22	16.80	14.20	2.81	0.00	3.97	1.32	2.29	0.00	5.65	2.99	2.84
	p (T<=t) one-tail	0.1438								0.2505								0.2461							
21.	2-Phenylethanol (1116)	-	-	-	-	-	-	-	-	0.00	0.47	0.16	0.27	0.00	0.64	0.21	0.37	0.00	0.99	0.33	0.57	0.00	1.34	0.50	0.73
	p (T<=t) one-tail	-								0.4402								0.1205							
22.	3.5-Dihydroxy-6-methyl-2.3-dihydro-4H-pyran-4-one1143	-	-	-	-	-	-	-	-	0.00	6.33	2.11	3.66	-	-	-	-	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	-								0.2113								-							
23.	4-Ketoisophorone (1147)	-	-	-	-	-	-	-	-	0.00	1.26	0.69	0.64	0.00	1.44	0.48	0.83	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	-								0.2805								-							
24.	Lilac aldehyde** (1157)	-	-	-	-	-	-	-	-	0.00	1.26	0.42	0.73	0.00	1.09	0.36	0.63	-	-	-	-	-	-	-	-
	P(T<=t) one-tail	-								0.4689								-							
25.	Terpinen-4-ol (1181)	0.00	5.61	2.32	2.36	0.00	3.85	2.26	1.67	0.00	0.32	0.11	0.18	0.00	0.24	0.08	0.14	0.00	3.66	1.22	2.11	0.00	12.99	4.33	7.50
	p (T<=t) one-tail	-								0.2113								0.2113							
26.	*p*-Cymene-8-ol (1185)	0.00	2.27	0.66	1.09	-	-	-	-	0.00	0.90	0.30	0.52	-	-	-	-	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	0.1554								0.2113								-							
27.	Myrtenal (1198)	0.00	1.87	0.47	0.93	0.00	1.53	0.79	0.64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	0.1784								-								-							
28.	Decanal (1208)	0.00	1.48	0.37	0.74	0.00	1.96	0.49	0.98	0.00	1.63	0.75	0.82	0.00	2.40	0.80	1.39	0.00	2.83	0.94	1.64	0.00	3.85	1.89	1.93
	p (T<=t) one-tail	0.1955								0.4594								0.1068							
29.	β-Damascenone (1380)	-	-	-	-	-	-	-	-	0.00	0.63	0.39	0.34	-	-	-	-	-	-	-	-	-	-	-	-
	p (T<=t) one-tail	-								0.0930								-							

8 ** - correct isomer is not identified; RI – retention indices in comparison with C_9-C_{25} alk

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Chemical alterations in honey ripened in beeswax adulterated with paraffin (PF-H)
- Beeswax adulteration affects the composition and quality
- Higher water %, lower glucose/water ratio in PF-H (susceptibility to fermentation)
- Higher acetic and citric acid content in PF-H; indication of incipient fermentation
- PF ripening media moderately changes the HS-VOCs chemical profile of PF-H