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Screening of the Honey Aroma as a Potential Essence for the Aromachology

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Abstract: The aim of the study was to determine the aroma profiles of four kinds of Slovak honey (sunflower, honeydew, acacia, and linden) by a qualitative and quantitative screening of their volatile compounds and by gas chromatography for the potential use in the aromachology and the business sphere. The results showed that several unique volatiles were identified in one kind of honey, while they were not identified in the remaining ones. The acacia honey had the unique volatile linalool oxide (1.13–3.9%); linden honey had the unique volatiles nerol oxide (0.6–1.6%), ethyl esters (0.41–8.78%), lilac aldehyde D (6.6%), and acetophenone (0.37%). The honeydew honey had the unique volatiles santene (0.28%) and cyclofenchene (0.59–1.39%), whereas 2-bornene (0.43–0.81%) was typical for sunflower honey. While linden honey was characterized by fruity ethyl esters, honeydew honey had more monoterpenoid compounds. In the principal component analysis model, the four kinds of honey could not be differentiated by aroma volatiles. However, it was possible to classify the linden and sunflower honey using the LDA. In conclusion, the current study provided experimental evidence that the marker compounds from different kinds of honey might be promising candidates for production of inhaling aromas.

Keywords: volatile organic compounds; scents; aromatization; bee product; gas chromatography

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1. Introduction

An aroma represents an effective marketing tool and is considered to be a new way of in-store communication with customers [1]. According to Horská et al. [2], nowadays the usage of fragrance compounds and essential oils in many business sectors is increasing. Many companies are using their own branded aromas in their shops, offices, or even during some marketing events or campaigns. More and more companies are creating aroma logos or corporate scents to be used indoors. Even retailers apply specific aromas to create comfortable environment where customers would spend more time and money by purchasing more products [3].

In general, these odors and aromas are distributed by installing aromatizers or diffusers in certain areas [4]. Moreover, aromatization may attract new customers; however, at first it is necessary to conduct aroma testing in order to select appropriate aromas prior to their implementation. For example, application of cappuccino aroma at store in Slovakia increased the volume of sales in confectionery category (desserts, chocolate bars, chocolates, and waffles) [5]. Furthermore; Berčík et al. [6] indicates that implementing aromatization in business spaces has positive impact on economic indicators, including the sector of services.

Aroma, as a marketing stimulus plays, an important role even in the food industry and gastronomy [7]. Honey, as a food product, has (besides health benefits and nutritional values) very specific characteristics from an organoleptic point of view. It is used not only in the food sector, but also in the cosmetic and pharmaceutical industries [8,9]. Healing properties of honey are used for massages, therapy wraps, or for production of scented candles. Honey contains compounds with antioxidant [10], antibacterial, antifungal, anticancer [11], and anti-inflammatory effects [8,9,11]. Compounds with such properties are mainly polyphenols [8,11]. However, these effects were confirmed also for some volatile organic compounds (VOCs), particularly terpenes [12,13]. VOCs from honey were previously determined by GC-MC analysis of simultaneous extraction and distillation extracts [14,15], HS-SPME/GC-MS [16–21], HS-GC-MS [22], and HS-SPME/GC×GC-TOF-MS [23,24] analyses. Even though honey diffusers are available on the market, there are no studies evaluating their impact on the business sphere.

The aim of this study was to characterize the aroma profiles of Slovak honey samples, in order to (i) find differences between four kinds of monofloral honey, and (ii) to determine promising volatile candidates for production of inhaling aromas.

2. Materials and Methods

2.1. Samples

In this study, a set of four kinds of honey (sunflower $n = 5$, acacia $n = 5$, honeydew $n = 5$, and linden $n = 5$) were analyzed (Table 1). The honey samples (300 g) were sourced from local Slovak small-scale beekeepers and were harvested in 2020. All the honey samples were stored at 20 °C and analyzed within six months after being harvested. Content of sugar was determined by refractometer designed to make corrections based on temperature using automatic temperature compensation (HR901, Krüss GmbH, Hamburg, Germany). A total of 20 samples (4 × 5) were analyzed in triplicate.

Table 1. Basic characterization of Slovak honey samples, including kind of honey, location, and sugar content.

Kind of Honey	Sample No.	Location (City)	Content of Sugar (°Brix [1] ± SD [2])
Honeydew	1	Kremnica	81.0 ± 0.0
	2	Sabinov	80.6 ± 0.0
	3	Nitra	80.8 ± 0.0
	4	Senec	81.6 ± 0.0
	5	Levoča	81.0 ± 0.0
Acacia	6	Choča	80.0 ± 0.0
	7	Oponice	81.2 ± 0.0
	8	Šala	79.0 ± 0.0
	9	Levice	80.2 ± 0.0
	10	Krupina	82.2 ± 0.0
Linden	11	Nitra	80.0 ± 0.0
	12	Zvolen	77.0 ± 0.0
	13	Krupina	78.2 ± 0.0
	14	Senica	78.8 ± 0.0
	15	Komárno	80.2 ± 0.0
Sunflower	16	Hlohovec	79.0 ± 0.0
	17	Oponice	79.0 ± 0.0
	18	Šala	78.6 ± 0.0
	19	Levice	81.8 ± 0.0
	20	Komárno	80.2 ± 0.0

[1] °Brix—Sugar content of an aqueous solution, where °Brix represents 1 g of sucrose in 100 g of solution. [2] SD, Standard Deviation ($n = 3$).

2.2. Determination of Volatile Organic Compounds

Sample preparation and isolation of VOCs were chosen based on a previous study by Kružík et al. [16]. VOCs were extracted from the honey samples using SPME fiber (DVB/CAR/PDMS 50/30 µm; Supelco, Bellefonte, PA, USA) with CombiPAL automated sample injector 120 (CTC Analytics AG, Zwingen, Switzerland). Each of the honey samples (2 g) was dissolved in a 20 mL glass vial using 2 mL NaCl solution in distilled water (200 g/L). The gas chromatography (Agilent GC7890B) with mass spectrometry (Agilent MSD 5977A) (Agilent Technologies Inc., Santa Clara, CA, USA) method equipped with the column HP-5ms (30 m × 0.25 mm × 0.25 µm; Agilent Technologies) previously described by Kružík et al. [16] was used for determination of VOCs in a modified version. A modified temperature program 40 °C (1 min), 5 °C/min, 250 °C (1 min) was used. Individual compounds were identified based on the comparison of the mass spectra with the commercial database of the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) mass spectral library (NIST17), and on the assessment of retention times with the literature [16,17]. The relative content (expressed in percentage) of determined compounds was calculated by dividing individual peak area by the total area of all peaks. Each sample was measured in triplicate.

2.3. Statistical Analysis

All of the data obtained were analyzed by descriptive statistics arithmetic average and standard deviation. To determine the aroma differences (>5% content) between the honey samples, descriptive statistics, normality tests, LDA (Linear Discriminant Analysis) and the PCA (Principal Component Analysis) were performed using the MS Excel and XLSTAT package program [25].

3. Results

By analyzing the saline solution of honey by GC-MS analysis, in total, ninety-four VOCs were identified, including alkanes, alcohols, alkenes, nitriles, acids, esters, monoterpenes, monoterpenoids, aldehydes, and ketones (Table S1). Several volatiles, that were present in one kind of honey and not in the other ones, can be marked as markers. The values shown in the Table 2 for each unique VOC are means of triplicate determinations with the standard deviation, retention time, and previous identification by the literature. As an example, the chromatograms of each kind of honey sample are shown in Figures S1–S4.

Table 2. Identified unique VOCs in four kinds of honey with percentage content [1] ± SD [2] and previous identification in literature [3].

Kind of Honey	Sample No.	Rretention Time (min)	Compound	Percentage Content (%)	SD	Literature
Acacia	9	10.5	Linalool oxide	1.13	0.15	[16–20,23,24,26–29]
	8		Linalool oxide	3.90	0.25	
	8	3.1	3-methyl-2-Butenal	0.86	0.08	[26,27]
	8	8.6	5,6-dimethylene-Cyclooctene	0.42	0.00	–
	6	23.9	2,6,10-Trimethyltridecane	0.48	0.14	–
Linden	13	13.4	Nerol oxide	0.60	0.07	[19,20,22,27]
	11		Nerol oxide	1.60	0.07	
	15	11.4	Linalyl acetate	0.56	0.23	–
	14	18.4	ethyl Nonanoate	0.57	0.24	[18,24,27]
	12		ethyl Nonanoate	1.30	0.25	
	15	13.8	Lilac aldehyde D	6.60	0.65	[14,16,21,23,26,30]
	12	19.6	ethyl Citronellate	5.17	0.37	–
	12	21.8	ethyl Decanoate	0.55	0.20	[15,19,20,22,24,27]
	12	20.2	ethyl Benzenepropanoate	0.41	0.07	[24]

Table 2. *Cont.*

Kind of Honey	Sample No.	Rretention Time (min)	Compound	Percentage Content (%)	SD	Literature
	14	13.9	ethyl Benzoate	7.63	2.48	[16,18–20,22,26–28,31,32]
	13		ethyl Benzoate	1.76	0.65	
	12	23.6	ethyl 4-isopropylbenzoate	0.43	0.13	–
	12	1.8	Ethyl acetate	8.78	3.40	[19,20,24,26,29–31,33,34]
	14	16.6	ethyl Benzeneacetate	1.70	0.29	[22,24]
	13		ethyl Benzeneacetate	0.43	0.04	
	12		ethyl Benzeneacetate	1.18	0.34	
	12	17.0	2-phenylethyl Acetate	0.55	0.08	[16,19,20,26,27]
	13	20.0	3,5-Dimethyl-2-octanone	0.71	0.16	[26]
	12	2.9	3-methyl-1-Pentanal	1.70	0.77	[24]
	13	4.0	3-methyl-1-Pentanol	0.59	0.11	–
	12		3-methyl-1-Pentanol	4.57	0.43	
	15	6.2	3-methyl-Pentanoic acid	1.16	0.31	[16,23,35]
	15	3.9	4,4-Dimethyl-3-oxopentanenitrile	3.76	0.54	–
	11	8.64	7-exo-ethenyl-Bicyclo[4.2.0]oct-1-ene	0.85	0.05	–
	13	10.2	Acetophenone	0.37	0.08	[15,24,27,28,36,37]
	15	1.6	Dimethyl sulfide	5.36	1.51	[17,24,29,32,37]
Honeydew	3	9.3	trans-beta-Ocimene	0.53	0.10	[19,20,23,27]
	2	21.9	Tetradecane	2.42	0.28	[23]
	4	4.9	Santene	0.28	0.12	–
	1	13.82	p-Mentha-1,5-dien-8-ol	0.59	0.05	–
	1		p-Mentha-1,5-dien-8-ol	4.80	0.48	
	2	15.0	Dodecane	1.99	0.29	[23,24,27]
	1	14.9	2-Propylphenol, methyl ether	0.52	0.08	–
	3	9.6	3-Carene	0.55	0.10	[20]
	3	2.0	3-methyl-Butanal	0.94	0.02	[17,24,29–35]
	4	16.4	4-(1-methylethyl)-Benzaldehyde	0.23	0.00	–
	1	8.58	alpha-Terpinene	0.57	0.04	[18–20,22,23,27,31]
	3	7.8	beta-Myrcene	0.92	0.20	[22,23]
	1	9.0	beta-Phellandrene	0.45	0.02	[30]
	1	7.82	Carveol	0.77	0.01	[23]
	1	20.5	Cosmene	0.84	0.09	[20]
	3	8.9	D-Limonene	1.67	0.36	[17–20,23,24,27,29,32]
	2	19.0	2-Methoxy-4-vinylphenol	0.21	0.02	[24,35]
	1		2-Methoxy-4-vinylphenol	0.41	0.00	
	1	6.7	2,4-Thujadiene	0.42	0.04	[22,23]
	3	4.3	3-Furanmethanol	0.79	0.28	–
	3	3.1	2,3-Butanediol	0.69	0.07	[19,23,32]
	3	7.4	1-Octen-3-ol	0.86	0.18	[21,31,32]
Sunflower	18	23.1	β-Calarene	2.75	0.90	–
	20	16.1	Cyclofenchene	1.20	0.37	–
	19		Cyclofenchene	0.64	0.04	
	18		Cyclofenchene	1.39	0.18	
	17		Cyclofenchene	0.59	0.09	
	16		Cyclofenchene	0.89	0.11	
	20	16.0	2-Bornene	0.68	0.42	–
	19		2-Bornene	0.43	0.12	
	18		2-Bornene	0.44	0.10	
	16		2-Bornene	0.81	0.27	

[1] The relative content (expressed in percentage) of determined compounds. [2] SD, Standard Deviation (n = 3). [3] Previously identified as VOCs in honey.

The PCA for four kinds of honey (Figure 1) revealed that 46.42% of the total variation embodied in 15 variables could be effectively condensed into, and explained, by the first two principal components (PCs), with eigenvalues of 4.3 and 2.7, respectively. The results show that the various kinds of honey contain characteristic volatile substances. On the other hand, there were no differences between the tested kinds of honey. The compounds as 3-methyl-1-pentanol, dimethyl ether, p-cymen-8-ol, lilac aldehyde D, and dimethyl sulfide are characteristic for linden honey. The acacia, sunflower, and honeydew honey samples were characterized by lilac aldehyde B, benzaldehyde, furfural, trans-linalool oxide, linalool, acetic acid, and hotrienol.

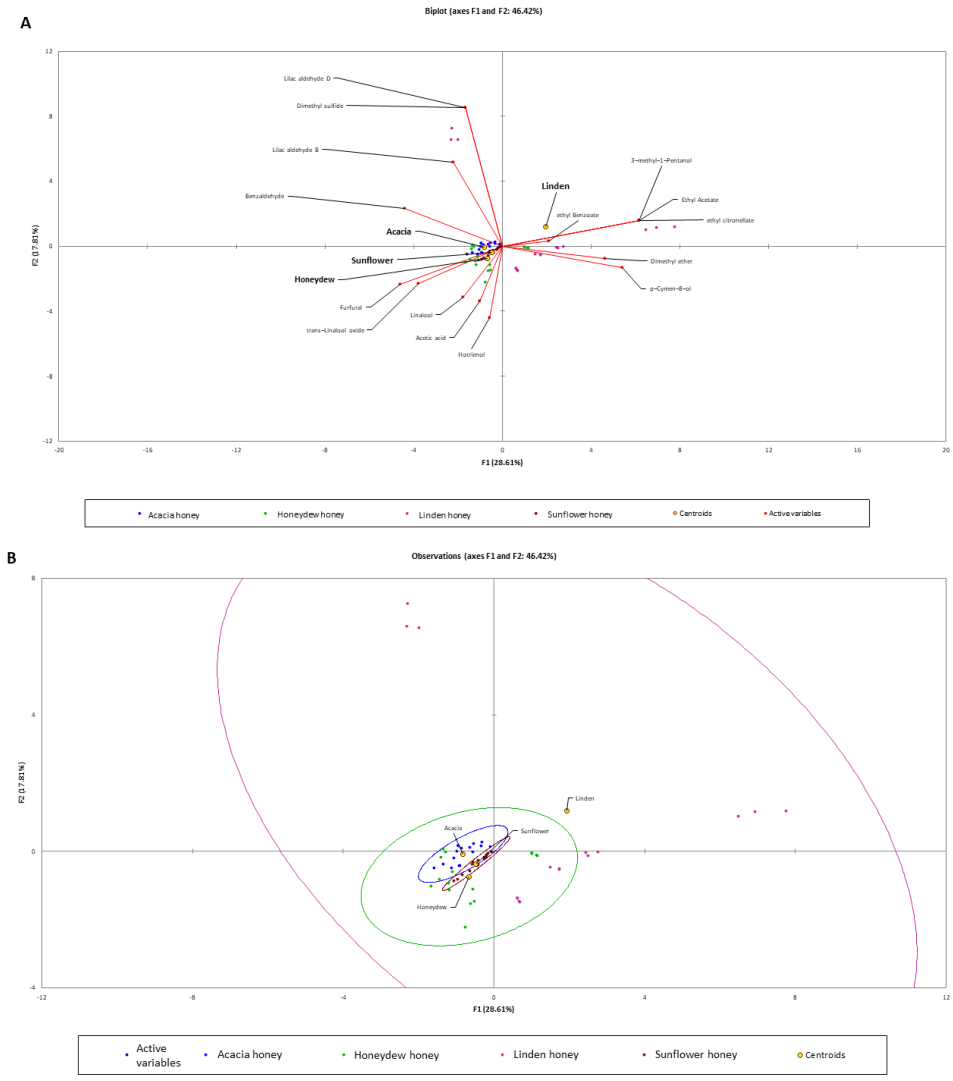

Figure 1. PCA evaluation of four kinds of honey (honeydew, acacia, linden, and sunflower); (**A**)—significant (>5%) aroma profile of samples with corresponding aromas; (**B**)—categorization of samples.

Discriminant analysis was used to classify the tested kinds of honey samples. The Wilks' Lambda test showed that the difference between the means vectors of the samples were significant ($p < 0.0001$). The first two eigen vectors explains 97.26% of variance (Figure 2). The confusion matrix calculated for the four tested samples was equal to 94.74% (data not shown). The Cross-validation: prior and posterior classification was performed to calculate the membership probabilities for unknown samples. The total accuracy of the cross-validation model was 85.96% (Table 3). Discriminant analysis can be used to classify the linden and sunflower kinds of honey but the acacia and honeydew kinds of honey were misclassified.

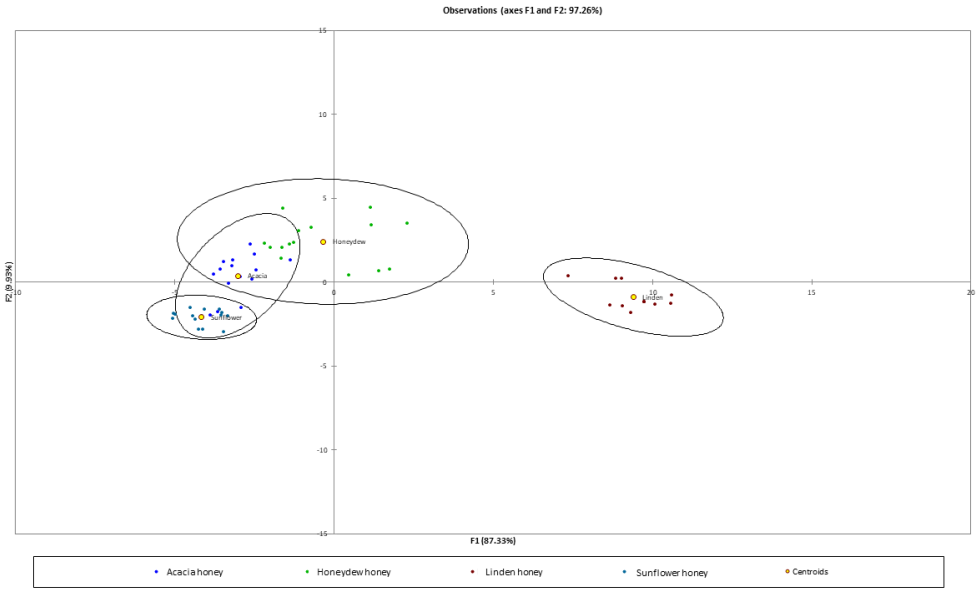

Figure 2. LDA map of kind of honey based on VOCs content.

Table 3. Confusion matrix for the cross-validation results.

from\to	Acacia	Honeydew	Linden	Sunflower	Total	% Correct
Acacia	10	1	0	4	15	66.67%
Honeydew	3	12	0	0	15	80.00%
Linden	0	0	12	0	12	100.00%
Sunflower	0	0	0	15	15	100.00%
Total	13	13	12	19	57	85.96%

4. Discussion

In total, 94 VOCs were detected in sunflower, acacia, honeydew, and linden honey samples. Some of these compounds have been previously reported by Plutowska et al. [26] in Polish linden, honeydew, and acacia honey. Sunflower and acacia honey samples from different countries were characterized by Radovic et al. [31]. The linalool oxide, as marker for acacia honey, is consistent with our results. The sunflower honey markers were α-pinene and 3-methyl-2-butanol according to Radovic et al. [31].

In this study, sunflower honey was characterized by β-calarene, cyclofenchene, and 2-bornene. Linden and honeydew had the most marker compounds. While tested linden honey was characterized by fruity ethyl esters [33], honeydew honey samples had more monoterpenoid compounds originated from aromatic plants [38]. The identified compounds in the tested honey samples were previously reported also in different kinds of honey [14–24,26–37], except linalyl acetate, which was previously tested for the fumigation of beehives to control the honey bee parasites [39]. Altogether, 16 volatiles have not been previously identified in honey samples.

According to The International Fragrance Association [40], 53 volatiles are recommended as honey fragrances. On the other hand, we identified honey volatile markers, which are not recommended as honey fragrances in the Fragrance Ingredient Glossary [40], but they are used in, for example, perfumery. The linden and honeydew samples contained anisyl alcohol (2-(para-anisyl) alcohol) (0.73%, and 0.80%, respectively), characterized as mild-floral, very sweet odor, reminiscent of lilac and vanilla with a faint, delicate, balsamic background used in perfumery [40].

The acetophenone is formed during the phenylpropane metabolism [37], and it was previously reported in chestnut honey [36,37]. However, this volatile was not previously considered by the research teams as a specific marker for the floral honey. It is characterized as a "floral" aroma fragrance described as: almond, orange, cherry, honeysuckle,

jasmine, and strawberry [40]. In the present work, the acetophenone was found in linden honey (0.38%).

Each tested sample had hotrienol which is known as probably being formed during the honey's ripening [41]. It was previously found in rapeseed honey from Czech beekeepers [16], leatherwood honey from Tasmania [41], and Greek citrus honey [42]. It could be the next honey fragrance, which is missing in the Fragrance Ingredient Glossary [40]. It can be used in many flavors, such as elderflower, grape, berry, and honey [43].

5. Conclusions

We confirmed that each kind of honey had its own unique volatiles, but the aroma differences by PCA in the tested monofloral honey samples were not confirmed. It is possible to classify linden and sunflower kinds of honey by using LDA. On the other hand, the LDA cannot clearly classify acacia and honeydew honey based on the selected VOCs. The reason may also be that the linden and honeydew honey samples have rich aromatic profiles and cover less aromatic honey samples. Studies evaluating their impact in the marketing or aromachology respectively were not published yet. According to our results, the anisyl alcohol, hotrienol, and acetophenone may be used as the honey fragrance.

In conclusion, the question arises as to whether customers would be able to recognize individual kinds of honey scents, and how this would affect their behavior.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11178177/s1. Table S1: Content of VOCs in four kinds of honey samples determined by HS-SPME GC-MS; Figure S1: Chromatogram of sunflower honey; Figure S2: Chromatogram of acacia honey; Figure S3: Chromatogram of honeydew honey; Figure S4: Chromatogram of linden honey.

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