LabWorks APPNOTE

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Comparison of Principal Component and Sensory Directed Analysis for the Differentiation of Aroma Characteristics of Honey from Different Botanical Sources

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Honey, Botanical Origin, Sensory Directed Analysis (SDA), Principal Component Analysis (PCA), Twister®, ThinFilm-SPME), Olfactory Detection Port

Abstract

Honey is an extremely popular natural food product created by bees from the nectar of various flowers and plants. These sources can impart a distinctive flavor and aroma to the honey. Some are more desirable to consumers than others, which enhances the value of those honey types. This study compares an analytical approach with principal component analysis (PCA) to a sensory directed analysis (SDA) approach for differentiating honey from multi-floral, wildflower, and clover sources.

Introduction

Honey is a natural food product, widely consumed both for its sweet taste, it's flavor and for medicinal benefits. Honey varieties have different aroma profiles due to several factors. These include the source of the nectar, geographical location, climate conditions, and the processing methods used. The distinctive flavors of honey are influenced by the nectar sources such as clover, wildflower, acacia, alfalfa, and buckwheat. These characteristics are recognized by consumers who often specifically look for them, thereby notably augmenting the value of honey compared to other sweeteners. Understanding the sensory characteristics of different botanical origins of honey can provide insight into the reasons for consumer preferences.

Principal Component Analysis (PCA) is a widely applied statistical method for simplifying large datasets and distinguishing sample differences. It has frequently been used to classify the botanical origins of honey by analyzing the volatile organic compounds (VOCs) in each sample [1-3]. While PCA efficiently identifies measurable distinctions between different sample types, it fails to offer sensory data about the differentiating VOCs. Compounds that significantly affect flavor often do not correspond to a discernable peak on the mass spectrometer. As PCA depends on these detectable peaks for data input, such information would be missing in this analysis. Furthermore, even if a sensory-active compound can be correlated with a detectable peak, it may not be present at a concentration above its odor threshold, thus not contributing to the overall aroma of the sample.

SDA is a process that utilizes gas chromatography in combination with the human nose and mass spectrometry to identify sensory-active compounds. The use of olfactory and MS detection enables the simultaneous determination of sensory-active regions of the chromatogram and mass spectral identification of the associated flavor compounds. As a result, SDA can be used to solve sensory-related challenges by determining the compounds responsible for producing desirable flavors in food products.

In this study, honey was analyzed to determine sensory differences related to botanical origin. An immersive TF-SPME/Twister® technique was used as a solventless means to extract and concentrate analytes from honey samples. The two approaches of PCA and SDA were used to differentiate the honey types and determine which provided the data necessary to distinguish the aroma profiles of the honey effectively.

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Experimental

Instrumentation

GERSTEL LabWorks Platform with CCD2, ODP 4 and Agilent 8890 GC/5977B Inert plus.

Analysis Conditions LabWorks Platform

CIS	
Pneumatics	Split (10:1)
Vent flow	50 mL/min until 0 min
Purge flow	10 mL/min at 0.01 min
Temperature	10 °C; 12 °C/sec; 280 °C (3 min)
TDU	
Pneumatics	Splitless
Temperature	40 °C; 720 °C/min; 250 °C (5 min)
Twister & TF-SPME	
Phase	PDMS & PDMS/HLB

Analysis Conditions Agilent 8890 GC/5977B Inert plus

Column	30 m Stabilwax-MS d_i = 0.25 mm d_f = 0.25 µm
Pneumatics	He; Pi = 13.3 psi Constant flow = 1 mL/min
Oven	40 °C (1 min); 10 °C/min; 250 °C (5 min)
MSD	Full scan, 40-350 amu

Sample Preparation

Honey samples were purchased from a local store. Honey types included clover, wildflower, and multi-floral honey of clover, berries, alfalfa, and wildflower. Approximately 1 mL of honey was transferred to a 10 mL screw-capped vial that was filled to volume with 9 mL of water. The honey was dissolved by vortex mixing for 30 seconds.

Extractions were performed on a 20-position GERSTEL Twister stir plate at 1100 rpm at room temperature. A conditioned PDMS Twister stir bar was added to each vial. A conditioned HLB/PDMS TF-SPME membrane was secured in a TF-SPME holder, and the holder was placed in the vial so that the TF-SPME was immersed in the sample. The samples were extracted for 1 hour at room temperature. The Twisters and TF-SPME membranes were removed, rinsed with water, and blotted dry before being placed together in a TD tube. The tubes were sealed with a transport adapter and placed in a 40-position tray on the MPS LabWorks Platform system for automated analysis.

Sample Introduction

Samples were desorbed in splitless mode in the Thermal Desorption Unit (TDU 2) with a 50 mL/min helium flow at 250 °C for 5 minutes. Analytes were trapped in the Cooled Injection System (CIS 4) PTV type inlet at 10 °C on a Tenax® TA liner. When desorption was complete, analytes were transferred to the GC column in split mode (10:1) by heating the inlet to 280 °C.

Olfactometry

The column effluent was split 2:1 between the Olfactory Detection Port (ODP 4) and MS, respectively. The ODP transfer line was heated to 250 °C. The mixing chamber was heated to 150 °C and purged with humidified nitrogen to prevent olfactory fatigue and nasal dehydration.

PCA Analysis

The MS data from the GC-O/MS analysis was used for PCA analysis. The PCA analysis was performed using the MetaboAnalyst Software [4]. Note that numerous peaks were detected but did not meet the match criteria for identification when searching against the NIST17 library. These peaks were assigned a number.

Results and Discussion

The PCA score plot in Figure 1 shows that the three honey types are well distinguished from one another, and the first two principal components account for 70% of the total variance in the samples. The multi-floral honey is differentiated from clover and wildflower along principal component 1, and the clover is differentiated from the other two varieties along principal component 2. The PCA loading plot, shown in Figure 2, arranges the compounds based on their strongest correlation with a particular sample. This indicates that they are most abundant in that sample and contribute significantly to the distinctions among the different types of honey. These compounds are listed in Tables 1-3. The relative peak areas are shown in each table, normalized to the relevant honey type.

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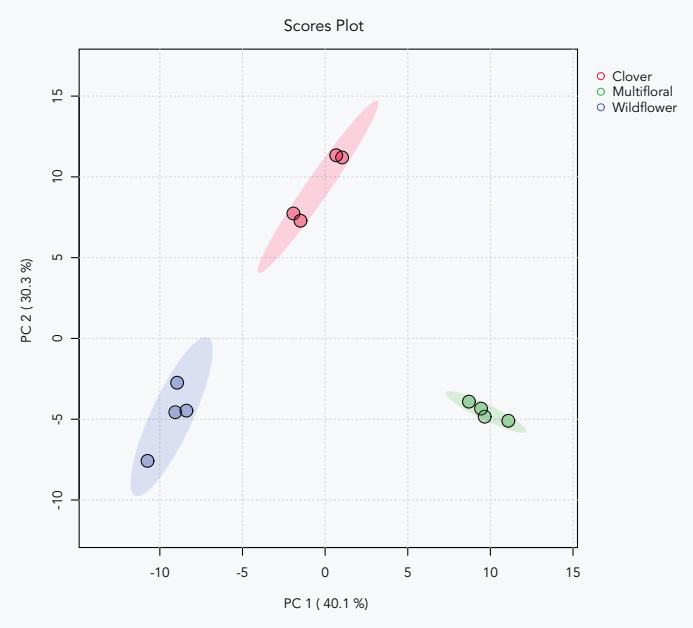

Figure 1: PCA score plot of honey samples from different botanical origins.

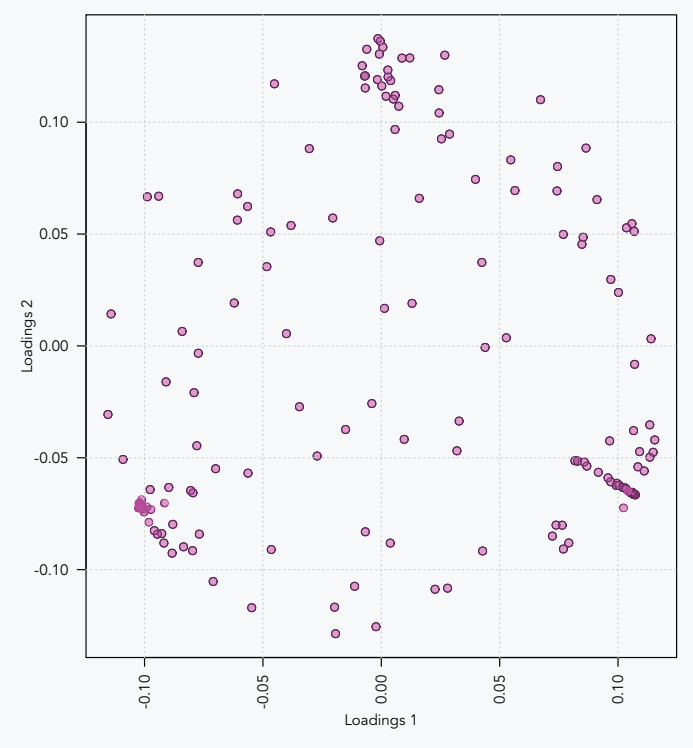

Figure 2: PCA loading plot of compounds.

Table 1: Compounds most relevant to the multi-floral honey with relative peak areas for n=4 and literature-based odor descriptors (n.d. = not detected).

Compound	M	W	C	Odor Descriptor from Good Scents Database [5]
Benzeneacetalde-hyde	100	10.4	27.1	honey, floral, rose
Coumarin	100	3.6	18.0	sweet, hay, tonka
Isophorone	100	n.d.	61.4	woody, green, camphor
33	100	0.5	12.6	
3,4,5-Trimethyl phenol	100	n.d.	34.9	phenolic
67	100	n.d.	11.7	
cis-Linalool oxide (furanoid)	100	10.7	21.9	earthy, floral, woody
alpha-Terpineol	100	n.d.	n.d.	pine, terpene, citrus
48	100	n.d.	n.d.	
8	100	n.d.	n.d.	
2-Ethylhexanoic acid	100	52.8	66.5	
47	100	n.d.	n.d.	
23	100	n.d.	n.d.	
54	100	n.d.	n.d.	
3-Methylbutanal	100	22.7	38.5	fruity, cocoa, fatty
6-Phenylisoquinoline	100	n.d.	n.d.	
69	100	n.d.	n.d.	
60	100	n.d.	n.d.	
36	100	n.d.	n.d.	
39	100	n.d.	n.d.	
63	100	n.d.	n.d.	
32	100	n.d.	n.d.	
Vanillin	100	20.4	17.5	vanilla
trans-Linalool oxide (furanoid)	100	n.d.	n.d.	floral
41	100	n.d.	n.d.	
N-Phenylformamide	100	n.d.	n.d.	
Phenylethyl alcohol	100	47.4	51.1	floral, rose
2-Phenylpropenal	100	n.d.	n.d.	green, hyacinth, lilac
2-Hydroxy-4-oxoiso-phorone	100	n.d.	n.d.	
64	100	n.d.	n.d.	
Ketoisophorone	100	40.8	27.1	musty, woody, tea, floral
Benzaldehyde	100	65.0	50.5	almond, cherry
9	100	63.5	n.d.	
beta-Eudesmol	100	68.1	54.6	woody, green

Table 2: Compounds most relevant to the wildflower honey with relative peak areas for n=4 and literature-based odor descriptors (n.d. = not detected).

Compound	M	W	C	Odor Descriptor from Good Scents Database [5]
2-Methoxy-4-vinyl-phenol	n.d.	100	36.8	clove, phenolic
beta-Damascenone	n.d.	100	63.4	apple, rose, honey
gamma-Terpinene	14.6	100	20.9	woody, terpene, citrus
Naphthalene	n.d.	100	n.d.	pungent, tarry
56	n.d.	100	n.d.	
1-Methylnaphtha-lene	n.d.	100	n.d.	naphthyl, medicinal, camphor
Benzyl salicylate	n.d.	100	n.d.	balsamic, herbal
34	n.d.	100	n.d.	
27	n.d.	100	n.d.	
p-Mentha-3,8-diene	3.9	100	n.d.	
Cinnamyl benzoate	n.d.	100	n.d.	balsamic, fruity
4	n.d.	100	n.d.	
46	n.d.	100	n.d.	
p-(1-Propenyl)-tol-uene	12.3	100	n.d.	
p-Mentha-1,3-dien-7-al	15.8	100	6.0	fatty, spicy
trans-3(10)-Caren-2-ol	13.4	100	4.8	
p-Cymene	17.3	100	-	citrus, terpene, woody
24	n.d.	100	n.d.	
Carvacrol	21.6	100	n.d.	woody, camphor, thyme
42	38.4	100	n.d.	
Cinnamyl alcohol	21.4	100	3.4	floral, powdery, cinnamon
Coumaran	36.2	100	26.8	
p-Cymen-8-ol	22.1	100	n.d.	fruity, cherry, floral
Cinnamaldehyde	39.5	100	7.8	cinnamon, spice, honey
30	53.0	100	n.d.	

Table 3: Compounds most relevant to the clover honey with relative peak areas for n=4 and literature-based odor descriptors (n.d. = not detected).

Compound	M	W	C	Odor Descriptor from Good Scents Database [5]
3-Methylcrotonitrile	n.d.	n.d.	100	
Ethyl 4-ethoxyben-zoate	n.d.	n.d.	100	
59	n.d.	n.d.	100	
2-Pentadecanone	n.d.	n.d.	100	fatty, spicy, floral
25	n.d.	n.d.	100	
Dimethyl disulfide	28.9	n.d.	100	sulfur, vegetal, onion
alpha-Pinene	18.7	17.7	100	woody, pine, earthy
2,4-Di-tert-butyl-phenol	48.1	51.8	100	
26	n.d.	n.d.	100	
2-Ethyl-1-hexanol	n.d.	n.d.	100	citrus, floral, oily
Menthofuran	n.d.	n.d.	100	musty, nutty, earthy
p-Xylene	n.d.	n.d.	100	
Methyl vanillate	n.d.	n.d.	100	spicy, phenolic, floral
Linoleic acid	n.d.	41.1	100	
Geranyl linalool	n.d.	n.d.	100	floral, rose
Octanoic acid	57.6	51.4	100	fatty, waxy, soapy

In a typical PCA workflow, GC-MS analysis is performed without an ODP. Consequently, no associated sensory information is available for the compounds detected. Therefore, when a compound can be identified, sensory information must be obtained from literature sources such as the Good Scents Company database [5]. Literature sources may be able to provide odor characteristics but can only determine whether that compound is present above its odor detection threshold with additional quantitative information. Tables 1-3 also list the odor descriptors from the Good Scents Company for the identified compounds.

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In the SDA approach, sensory and chromatographic information are generated simultaneously to enable data correlation and definitive identification of sensory-active compounds. First, a sensory panel determines the key sensory characteristics of each honey before extraction and subsequent GC-MS/O analysis. While each honey had characteristic honey/floral notes, additional aromas were distinct to each honey type. The multi-floral honey had caramelized brown spice and phenolic characteristics. The wildflower honey was described as fruity/apple, hay/barnyard, waxy, woody, brown spice, and minty. The clover honey had notes of peach, green, herbal, and fatty acid.

Tables 4-6 list the key odors detected at the ODP for each sample. The tentatively identified compound column indicates compounds that eluted during the time window of the odor detected. A reference standard should be analyzed under the same instrument conditions to confirm identification. Compounds also found using PCA analysis (Tables 1-3) are marked with an asterisk. While there are many commonalities in the data generated from the two approaches, there are several differences too.

Table 4: Key aromas detected at the ODP for multi-floral honey.

Start RT [min]	Stop RT [min]	ODP Descriptor	Tentative Compound (s)
3.99	4.12	malty, cocoa	3-Methylbutanal*
8.71	8.80	cotton candy	
9.80	9.93	floral, honey, green	Benzeneacetaldehyde*
9.93	10.02	cotton candy	
10.89	10.96	floral, rose, honey	Phenyl ethyl alcohol*
11.49	11.58	floral	2-Phenylpropenal*
12.72	12.77	anise	
12.90	13.04	floral, honey	Phenylacetic acid
13.69	13.77	floral	Cinnamyl alcohol
13.80	13.85	brown spice	3,4,5-Trimethylphenol*
14.33	14.43	woody, brown spice	33*
15.02	15.16	vanilla	Vanillin*
15.57	15.73	musky, woody, spice	Coumarin*

*Compound also identified with PCA analysis

Table 5: Key aromas detected at the ODP for wildflower honey.

Start RT [min]	Stop RT [min]	ODP Descriptor	Tentative Compound (s)
6.60	6.69	fruity, red fruit, tropical	
8.34	8.39	fruity	
9.09	9.16	floral, fruity, green	
9.68	9.75	cotton candy, honey	
9.80	9.95	honey, floral, rose	Benzeneacetaldehyde
10.32	10.37	brown spice, maple	
10.84	10.87	herbal, minty	
10.90	10.98	floral, rose	Phenyl ethyl alcohol
11.64	11.74	musty, waxy	Octanoic acid
12.06	12.14	musty, barnyard, mothballs	Naphthalene*
12.74	12.77	anise, herbal	
12.85	13.06	floral, honey	Phenylacetic acid
13.53	13.56	floral, mint	p-Mentha-1,3-dien-7-al*
13.71	13.76	floral, honey	p-Mentha-3,8-diene* Cinnamyl alcohol*
13.83	13.86	brown spice, clove	2-Methoxy-4-vinylphenol*
14.06	14.10	waxy	
14.30	14.36	minty	35
14.37	14.48	brown spice	
14.74	14.81	fruity, apple	beta-Damascenone*
14.87	14.92	musty, barnyard	
14.99	15.06	vanilla	Vanillin
15.57	15.66	musky, brown spice	Coumarin
15.68	15.73	musty, woody	42*
16.83	16.97	musty, waxy, soapy	
18.48	18.55	woody, minty	56*
19.18	19.28	honey, brown spice	

*Compound also identified with PCA analysis

Table 6: Key aromas detected at the ODP for clover honey.

Start RT [min]	Stop RT [min]	ODP Descriptor	Tentative Compound (s)
5.04	5.12	vegetal	Dimethyl disulfide*
6.40	6.52	sweaty, musty, fatty acid	
6.61	6.69	fruity	
9.11	9.16	green, herbal, floral	
9.81	9.88	floral, rose, honey	Benzeneacetaldehyde
10.66	10.71	green, herbal	Nonanal
10.89	10.98	floral, rose	Phenyl ethyl alcohol
11.42	11.49	green, vegetal	Lilac aldehyde
12.18	12.25	floral, fresh	decanal
12.65	12.72	green, herbal	25*
12.86	12.96	floral, honey	Phenylacetic acid
14.74	14.80	fruity, berry	beta-Damascenone
16.49	16.54	fruity, peach, creamy	Ethyl 4-ethoxybenzo-ate*

*Compound also identified with PCA analysis

The compounds benzeneacetaldehyde, phenyl ethyl alcohol and phenylacetic acid are common to all three honey types, and their aromas at the ODP are described as floral, rose, and honey-like. These have been previously reported as being ubiquitous to all honey types and contribute to the characteristic honey aroma [6]. However, the PCA analysis determined that the presence of benzeneacetaldehyde and phenyl ethyl alcohol were distinguishing characteristics of the multi-floral honey. This can be misleading as the PCA only considers peak signal intensity and not the odor threshold of a compound. Given that these are low odor threshold compounds, they will contribute to the honey flavor regardless of the intensity differences seen in these samples.

For the peaks that could not be identified, no further information could be gathered in the PCA approach. However, in the SDA approach, sensory information can still be obtained using the ODP. Many of these compounds have key odor descriptors that are important to distinguish the botanical types. For example, unknown 33 was described as woody and brown spice in the multi-floral honey, and unknown 56 was noted as woody and minty in the wildflower honey. This sensory information greatly increases the efficiency of the analysis by letting the analyst know which unknowns warrant identification.

There are several areas where an odor of interest was detected at the ODP, but no peak signal was seen. For example, at 6.40 minutes in the clover honey, a sweaty, fatty acid note was smelled, but no peak signal was seen. This is not a common odor descriptor for honey and could be an off-odor indicative of fermentation in the honey [7]. This was the only fatty acid odor detected at the ODP for this sample. These are low-odor threshold compounds that can be smelled at concentrations below the instrument detection limit. Since consumers adversely react to off-odors, which ultimately affects the market value of the product, it is important to be able to identify these types of compounds. More mass on column can be achieved by utilizing the multi-desorption mode in the GERSTEL Maestro software [8] or selective trapping at the ODP [9] to obtain a peak signal for identification. Because of the lack of chromatographic signal, there is no measurable information to input for the PCA analysis. As a result, these key odors will be completely missed in the PCA approach. The PCA approach only focuses on identifying patterns and relationships within the identifiable data without explicitly providing a direct link between specific compounds and their respective sensory attributes.

Conclusion

This research emphasized the contrast between the insights offered by the two data analysis approaches. The PCA technique is a comparatively swift and straightforward method for differentiating between types of honey, which makes it most useful for quality assurance and identifying potential product adulteration. In contrast, the SDA approach provides a much more detailed analysis, accurately identifying the botanical origin of essential sensory-active compounds. When this information is integrated with customer feedback, it can prove highly beneficial for both quality assurance and the development of new products.

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