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Differentiation of alpine rose honey based on volatile compounds

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Functional Plant Compounds

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Declaration of Independence

I hereby declare, that no other aids and literature sources than those mentioned were included. The crucial work was carried out by myself and all those involved were credited with their contribution to the work. The diploma thesis submitted for assessment was written independently. The thesis was not submitted or published elsewhere.

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Index of Abbreviations

CHC ..... *cuticular hydrocarbon*
GC-MS ..... *gas chromatography/mass spectrometry*
SPME ..... *solid phase microextraction*
USE ..... *ultrasonic solvent extraction*

Abstract

Alpine rose honey or “Almrauschhonig” is a speciality among the honey varieties. It is gathered from plants of the *Rhododendron* family including *Rhododendron ferrugineum* and *Rhododendron hirsutum*, native to the Central European Alps. Different honey types such as blossom honey, honeydew honey, and mountain honey are compared in this diploma thesis to alpine rose honey. To distinguish the mentioned types of honey some quality parameters (such as pH-value, free acidity and electrical conductivity) were determined. Furthermore, honey was distilled by hydro distillation and the essential oil compounds determined by gas chromatography/mass spectrometry (GC-MS). The alpine rose samples showed a strikingly low pH-value (4.1) and low electrical conductivity (381 μS/cm) but did not differ in these parameters from blossom honey. Four compounds were detected in the essential oil which distinguished alpine rose honey from the other samples. These include 2-pentyl furan, *cis*-linalool oxide, decanal and carvomenthenal. The blossom honey samples presented five compounds which allowed a distinction from the other honey samples such as α-eudesmol, 6,10,14-trimethyl-2-pentadecanone, 9-eicosyne, nonacos-1-ene and decyl hexadecyl ester carbonic acid. The honeydew honey only showed one component to be distinguished from the other honey samples and that was borneol. The mountain honey samples, on the other hand, displayed no significant compounds by which they could be distinguished from other honeys; however, they revealed a similar compound pattern to those of the alpine rose honeys and the honeydew honeys. Using discriminant analysis, the honey samples could be distinguished from one another.

Abstrakt

Alpenrosenhonig oder “Almrauschhonig” ist eine Besonderheit unter den Honigsorten. Er wird aus Pflanzen der *Rhododendron*-Familie gewonnen, darunter *Rhododendron ferrugineum* und *Rhododendron hirsutum*, die in den mitteleuropäischen Alpen beheimatet sind. Verschiedene Honigarten wie Blütenhonig, Waldhonig und Gebirgshonig werden in dieser Diplomarbeit mit Almrauschhonig verglichen. Zur Unterscheidung der genannten Honigsorten wurden einige Qualitätsparameter (wie pH-Wert, freie Säure und elektrische Leitfähigkeit) bestimmt. Darüber hinaus wurde Honig durch Hydrodestillation destilliert und die ätherischen Ölverbindungen durch Gaschromatographie/Massenspektrometrie (GC-MS) bestimmt. Die Almrauschproben wiesen einen niedrigen pH-Wert (4,1) und eine geringe elektrische Leitfähigkeit (381 μS/cm) auf, unterschieden in diesen Parametern jedoch nicht vom Blütenhonig. In den ätherischen Ölen wurden vier Verbindungen nachgewiesen, die Almrauschhonig von den anderen Proben unterschieden. Dazu gehören 2-Pentylfuran, *cis*-Linalooloxid, Decanal und Carvomenthenal. Die Blütenhonigproben enthielten fünf Verbindungen, die eine Unterscheidung ermöglichten, darunter a-Eudesmol, 6,10,14-Trimethyl-2-pentadecanon, 9-Eicosin, Nonacos-1-en und Decylhexadecylester-Kohlensäure. Der Waldhonig wies nur einen Bestandteil auf, der sich von den anderen unterschied, und zwar Borneol. Die Gebirgshonigproben hingegen wiesen keine signifikanten Verbindungen auf, durch die sie von anderen Honigen unterschieden werden konnten; sie zeigten jedoch ein ähnlich zusammengesetztes Muster wie die Almrauschhonige und die Waldhonige. Mittels Diskriminanzanalyse konnten die Honigproben voneinander unterschieden werden.

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1. Introduction

Honey is a food product produced by honeybees (*Apis mellifera*). Besides its good taste, some honey varieties are also used in medicine for its antibacterial and anti-inflammatory effects.

The most important starting material for the honey preparation is the nectar of flowers or sweet excretory products from various insects, the so-called honeydew (1,2). After collecting nectar and honeydew, the product is brought to the beehive where, under many steps, it is converted into honey. Due to the various origins of the honey, it can be differentiated e.g., in blossom honey, honeydew honey.

Volatile compounds, which are partly responsible for honey's flavours can be indicators for honeys' origin. The flavour is mainly influenced by sugar and aroma components of the plant from which the bees gather the nectar. Flower nectar is more or less 'impregnated' with the plant's own fragrances, with the result that blossom honeys in general, possesses more aroma components than honeydew honeys (3).

1.1. Honey and its Compounds

The main chemical ingredients of honey are water and sugar, in particular fructose and glucose, which are estimated at around 95% (consisting of 75% monosaccharides along with 10-15% disaccharides and small amounts of other sugars) (4,5). Other ingredients include amino acids, proteins, enzymes, hormones, lipids, phenolic compounds, vitamins, essential oils, pigments, sterols, phospholipids and mineral salts (2). While organic acids, enzymes, proteins and volatile compounds are just minor constituents, they are ensuring the unique taste in different honeys. Besides having a big impact on taste, the compositions also indicate from which plants and therefore, from which area the nectar and honeydew is harvested.

The sugar content in honey is responsible for its hygroscopic properties and viscid consistency. Furthermore, the antimicrobial traits arise due to the sugar concentration, low pH, hydrogen peroxide, flavonoids, phenolics and terpenes (6).

Due to its good flavour and its health-enhancing abilities, honey in general, but mostly monofloral honeys have increased its commercial value. Therefore, adulterations related to the nutritional value of the honey, or the mislabelling of the botanical origin may occur. To

identify such adulterations, different analytical methods, such as aroma profiles or pollen analysis, are used to guarantee the quality of honeys. Aroma profiles give insight into the organoleptic quality and authenticity of honey. Hence, they are often used to evaluate the characteristics of different honey samples. Due to the high number of volatile compounds, the aroma profile is equivalent to the “fingerprint” of a product which can also be used to determine the products origin (1,7,8).

To identify which compounds have a main impact on the smell and taste of the honey samples, distillation was used to isolate them. After extracting the essential oils from the honey, the samples were analysed with GC-MS. The resulting aroma profiles provided insight into the existing essential oils from the various honey samples.

1.1.1. Electrical conductivity and pH Value

Besides the aroma profiles, which are among the most typical features for food evaluation of both organoleptic quality and authenticity, (4) there are other indicators which give information about the quality and type of the honey.

Honeydew honeys can be distinguished from blossom honeys for example by electrical conductivity.
Honeys with an electrical conductivity of higher than 800mS/cm are classified as honeydew honeys, and those below as blossom honeys.
The pH of honeys depends on a few parameters, such as organic acids, inorganic ions, minerals ionized as well as the botanical source, nectar and/or honeydew. It is also associated with the stability and shelf life. In general the, pH-value of honeys lies between 3.3 and 5.5 (2,9).

Other than these two measurement methods, the organoleptic factors, such as colour, smell and taste also have a big impact on the classification. The colour ranges from almost colourless to dark brown (9). For example, honeydew-honey is dark yellow or brown and has a very spicy and aromatic smell, while flower honey has a light-yellow colour and a sweet smell. The darker colour of the honeydew honey is mainly due to its mineral and phenolic contents and other colour compounds. Also, the flavour of honeydew honeys is more intense than that of floral honeys. Then again, blossom honey is sweeter than honeydew honey (2).

1.2. What is Alpine rose honey

Nectar from Alpine rose honey is gathered from the flowers of *Rhododendron ferrugineum* and *Rhododendron hirsutum*. They belong to the genus of *Rhododendron*, which is a common plant genus of the northern Hemisphere and North-East Australia. The evergreen plant is growing at coasts and in the highlands and the most representative of this genus are found in Asia. In Asia, North America and Europe, the plant is used in traditional medicine. Besides that, the plant is also known for its toxicity which made it a popular poison (10).

The *Rhododendron* species growing wild in Austria are *Rhododendron ferrugineum* and *Rhododendron hirsutum* as well as their hybrid *Rhododendron* intermedium. These plants are used in traditional medicine for anti-inflammatory treatment. Honey gained from alpine roses is characterized by an underrepresentation of pollen (30-60%) and also by its very light colour, low conductivity and ash values (11).

Besides its phytotherapeutic effect, the genus *Rhododendron* is also well-known for its toxicity due to the occurrence of grayanotoxins. There have been cases that documented honey intoxication, also known as “mad honey poisoning”, which is prevalent in the black sea area, where *Rhododendron luteum* and *Rhododendron ponticum* are growing. The grayanotoxins are toxic polyhydroxylated diterpenes which target voltage-dependent sodium channels of excitable cells. The severity of the symptoms is dependent on the dose and can be as mild as gastro-intestinal symptoms up to complete atrioventricular block (10). Due to the parasympathetic stimulation, it can be treated with saline infusions and atropine.

1.3. Essential oils in honey

Essential oils are a mixture of volatile, liposoluble compounds obtained by hydro-distillation. The impact of essential oils on the human body works, on one hand, by smelling the product’s scent and, on the other hand, through their pharmacological components (12). The development of essential oils occurs mainly in adolescent plants. Essential oils are cytotoxic to the plants. Therefore, they are stored in particular spaces which are in or close to the oil forming parts of the plant. The oil forming parts of plants are already modelled in the bud stage, hence, the amount of oil forming properties only decreases the older the plant gets. The storage location of essential oils is dependent on its origin and can be found in oil cells, intercellular excretory spaces, glandular hair, glandular scales and milk tubes (13).

Honeybees gather the nectar from these plants and convert it into honey, where afterwards, the essential oils can be found. In general, the most common ingredient of essential oils in plants are *terpenes,* less common are *phenylpropanes*. It is also established that terpenes and norisoprenoids originate from the nectar and not from the bees and the comb environment (14).

Due to the heat impact of the distillation process on sugars and amino acids, more thermal artefacts are being generated. Furthermore, sensitive compounds are easier decomposed and new compounds can emerge while low volatile and water soluble compounds cannot be quantitatively determined (15–18).

2. Material and Methods

2.1. Honey samples

Different floral and honeydew honeys from beekeepers or specialised shops in Austria were purchased. In total 49 honey samples were used. The chosen samples included 13 flower honeys, 11 forest honeys [honeydew honeys], 8 mountain honeys, 7 flower-honeydew honeys and 10 alpine rose honeys. The samples were labelled with an internal scheme (e.g., flower honey 1 = BL01). They were stored at room temperature. Some honeys were crystallized over the storing process. They were liquefied in a waterbath at 30°C before analysis.

2.2. Electrical conductivity

To measure electrical conductivity 20g (dry weight) honey was weighed in. To obtain the dry weight, water content had to be determined first. Water content was determined with a honey refractometer which was calibrated with calibration fluid (both from Hong Han GmbH, Heidelberg, Germany). After water content was determined dry weight was calculated.

$$\text{dry content } [\%] = 100 - water\ content\ [\%]$$

To get exactly 20g of dry weight, another formula had to be used to get the exact amount of honey which had to be weighed in and is equivalent to 20g dry weight.

$$\text{dry weight } [\text{g}] = \frac{20\ [\text{g}]}{\text{dry content } [\%]} * 100\ [\%]$$

The calculated dry weight was then dissolved with 100mL MilliQ water. After dissolving the honey in the MilliQ water the conductivity meter was held into the solution and the conductivity was displayed. The unit of the electrical conductivity is mS/cm.

To calibrate the conductivity meter 0.01M KCl was used.

The conductivity meter used was an Ino Lab, Cond Level 1 (WTW GmbH, Weilheim, Germany).

2.3. pH Value and content of free acids

The pH-meter used was a Product edge Multiparameter pH-meter (Hanna Instruments, Vöhringen, Germany). For the measurement of the pH-value 10g of honey was weighed in, filled up to 75mL with MilliQ water and mixed. The solution then was placed on a magnetic stirrer and the initial pH-value was measured. After its determination 0.2mL of 0.1M sodium hydroxide at a time was added until the pH-value reached 8.3. The amount of 0,1M sodium hydroxide was then multiplied by 10 and the result is the content of free acids.

2.4. Distillation

For distillation a distillation modified Clevenger-apparatus was used. 200g of honey were weighed and mixed with approximately 400mL of MilliQ water and distilled for 3h. 1mL Hexane was used as a solvent to collect the essential oil.

2.5. GC-MS

The GC-MS was an Agilent 7890A coupled to a 5957C VL MSD (Agilent Technologies, Santa Clara, USA). The ion source was an electron impact source, source temperature was 230°C, quad temperature was 150°C and type of acquisition was scan.

Separation was carried out on an HP-5MS Phenyl Methyl Silox of 30m length and 250μm internal diameter with a layer thickness of 0,25μm. The flow was constant at 2mL/min with He as carrier gas. The oven temperature started with initial temperature 40°C, heated with 5°C/min until 100°C and then with 10°C/min until reaching 320°C. The injection volume was 1μl with a split ratio of 1:2. The thermal Aux 2 (transfer line into the mass selective detector) temperature was programmed at 280°C.

2.6. Qualitative and Quantitative Determinations

The compounds were identified comparing their experimentally obtained mass spectra with the compound spectra available from the NIST database (Wiley Registry 12th Edition / NIST 2020 Mass Spectral Library). Additionally, compounds needed to meet published linear retention indices, calculated based on the retention times of a homologous series of n-alkanes C9-C29 (Merck KGaA, Darmstadt, Germany).

2.7. Statistics

For the statistics evaluation the Program R (R Core Team, 2023) under RStudio 2023.09.0 (RStudio Team, 2020) with the packages FactoMineR and factoextra was used.

3. Results

3.1. Quality Parameters

The quality parameters gave previous information about some characteristics of the honeys. Those differed a lot in the different honey groups. Once the quality parameters were examined, three honey samples were excluded because honey characteristics proofed their labelling wrong.

Table 1: pH, free acidity, water content and electrical conductivity of 49 honey samples of Austrian origin

sample	pH	free acidity [mEq/kg]	water content	electrical conductivity [mS/cm]
AL01	3.92	16	16.2	205
AL02	4.07	12	15.6	230
AL03	3.95	35	15.6	439
AL04	4.11	20	16.4	300
AL05	4.09	20	16.4	276
AL06	4.30	34	16.2	782
AL07	4.20	18	15.8	336
AL08	4.11	39	16.4	684
AL09	4.00	16	16.4	258
AL10	3.92	23	15.0	299
BL01	4.13	23	15.0	305
BL02	4.40	25	15.4	534
BL03	4.28	16	15.2	296
BL04	4.00	20	15.8	243
BL05	4.21	22	15.2	417
BL06	4.47	16	16.0	436
BL07	4.06	24	16.6	395
BL08	4.02	23	16.0	317
BL09	4.19	14	15.4	188
BL10	3.91	21	17.8	280
BL11	4.03	22	15.6	414
BL12	3.87	27	16.4	437
BL13	4.76	32	16.4	1058

GE01	4.39	34	16.4	812
GE02	4.66	31	15.0	952
GE03	4.59	20	16.0	647
GE04	4.30	45	18.6	954
GE05	4.56	28	16.2	858
GE06	4.15	17	16.8	243
GE07	4.05	18	15.2	215
GE08	4.89	34	14.6	1306
WA01	4.89	34	15.2	1190
WA02	4.69	31	15.6	819
WA03	4.81	28	16.2	866
WA04	4.81	33	15.4	1053
WA05	4.82	18	15.8	989
WA06	5.08	31	15.4	1318
WA07	4.92	34	16.0	1152
WA08	4.99	38	15.2	1387
WA09	4.66	33	15.8	813
WA10	4.89	33	15.2	1008
WA11	4.74	38	16.4	1014
WB01	4.65	24	15.8	861
WB02	4.65	32	15.2	990
WB03	4.70	22	15.8	731
WB04	4.45	32	15.8	743
WB05	4.45	32	15.4	758
WB06	4.21	32	16.2	545
WB07	4.31	20	16.4	388

3.1.1. pH-value

The pH values of all honey samples are listed in Table 1. The range of the pH-values lied generally between 3.3 to 5.5 depending on its origin (2,9). The blossom honeys (BL01 – BL13), even though mixed and not monofloral, ranged from 3.87 to 4.76 with an average of 4.18.

The honeydew samples (WA01 – WA11) ranged from 4.66 to 5.08 with an average of 4.85.

The alpine rose honey group (AL01 – AL10) ranged from 3.92 to 4.30 with the mean pH at 4.07. These results were all in the range of the blossom honeys.

The mountain honey samples (GE01 – GE08) ranged from 4.05 to 4.89 with a mean pH of 4.45. These honeys are also a mixture of blossom and honeydew honeys, which can be seen in the pH-values.

The blossom-honeydew honeys (WB01 – WB07) ranged from 4.21 to 4.70 with an average of 4.49.

Comparing the results in Table 1, the difference between honeydew honey and the other types of honey were clear. Blossom honey in general scored the lowest range in pH-value, whereas honeydew honey scored the highest. The other groups of honeys varied within this range but since they were mostly a mixture of honeydew and flower honey it is understandable.

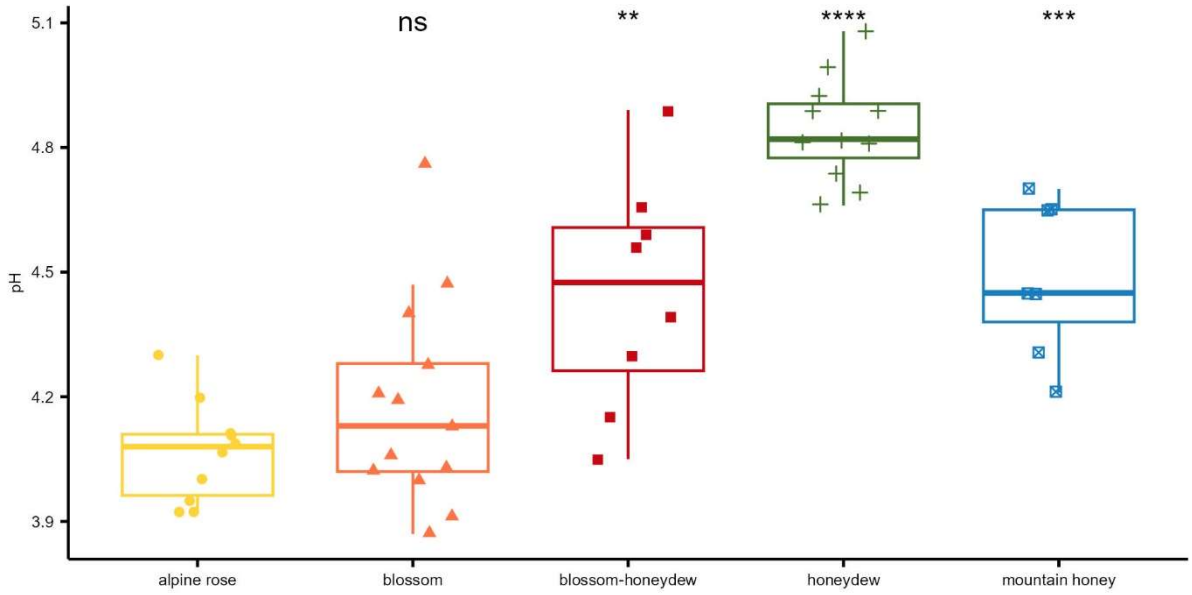

Figure 1: Boxplot of the pH of the different honey types compared to the alpine rose samples

In Figure 1 the pH-values of the honey samples are compared which shows that there is no significant difference between the alpine rose samples and the blossom samples. Since alpine rose honey is also mainly a blossom honey from higher regions it is reasonable that these two do not differ much. However, it is noticeable, that the mean pH of the alpine rose honeys is even lower pH than the mean pH of the blossom samples.

There is a significant difference between alpine rose honeys and blossom-honeydew honeys and a high significant difference between alpine rose honeys and mountain honeys. The biggest difference is between alpine rose honeys and honeydew honeys where there is a very high significant difference in the pH-values.

3.1.2. Free acidity

The acidity of honey is caused by organic acids (tartaric, citric, oxalic , acetic, etc), nectar or bees secretion (19). It is analysed by titration with potassium hydroxide. This parameter is also related with the deterioration of honeys. The amount of free acids in honeys is stipulated in the honey regulations and is not allowed to exceed 50 milliequivalents of acid per kg [mEQ] (20). If the values surpass 50 [mEQ] it could be an indication of fermentation of sugars into organic acids. At the same time, geographical origin, harvesting season and the presence of different organic acids can affect the honeys' acidity (5).

In Table 1, the honeys' free acidity was presented. The lowest value was at 14 mEq/kg and the highest at 32 mEq/kg. The highest value is from sample BL13 which also has a high pH for a blossom honey. It must be considered that this honey was falsely declared as blossom honey but was actually a honeydew honey. If BL13 is excluded, the next highest value is 27 mEq/kg which kept the range of the free acidity on a small scale.

The honeydew samples in general have higher values ranging from 18 to 38 mEq/kg.

In the alpine rose samples, it is noticeable that the highest and lowest values even exceeded the range of the blossom and honeydew samples. They range from 12 to 39 mEq/kg.

The mountain honeys ranged from 17 to 45 mEq/kg. GE04 was with 45 mEq/kg extraordinarily high.

The blossom-honeydew honey samples ranged from 20 to 32 mEq/kg with no further outliers.

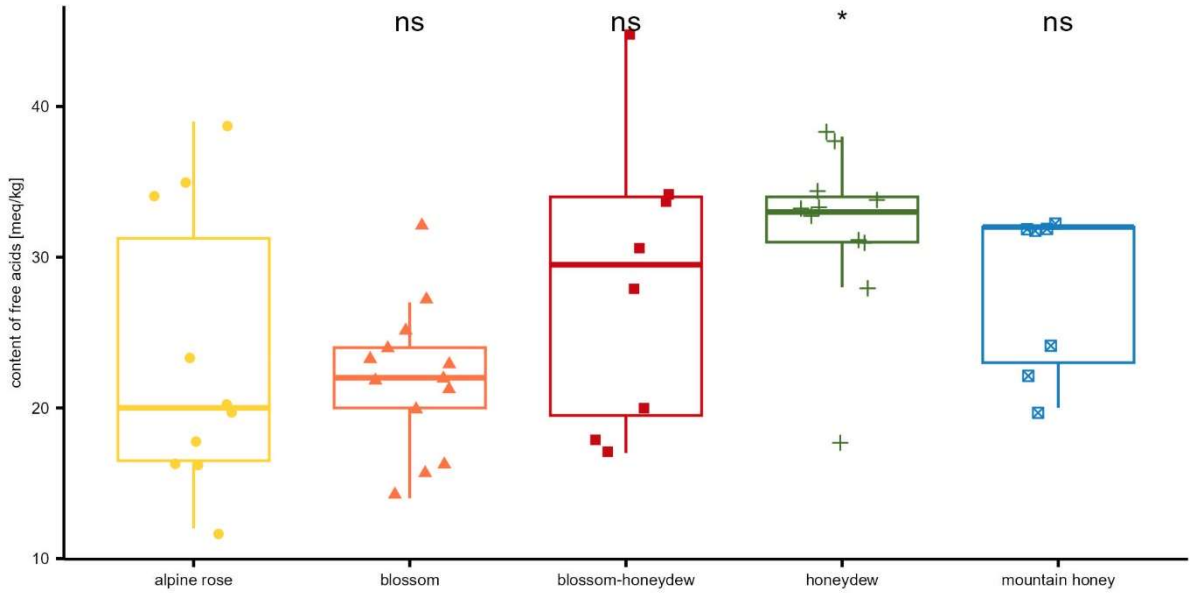

Figure 2: Boxplot of the free acidity of the different honey types compared to the alpine rose samples

The honey samples did not differ a lot in free acidity. There was only a significant difference between the alpine rose samples and the honeydew samples.

No significant difference between alpine rose honeys and the other honey samples was established. Noticeable is that the range of the honeydew honeys and also of the blossom honeys was very narrow.

3.1.3. Water content

The amount of water in honey is an important factor which influences the honey's stability fermentation during storage. Besides the sugars, it is the second largest fraction of honey. The water content is also a relevant factor influencing physical properties such as viscosity and crystallization, just as colour, flavour taste, specific gravity, solubility and conservation (5,21).

Honey with high contents of water ferments easily with time (22). Therefore, the type of honey (flower or honeydew) does not give an insight into the amount of water in the honey more than the harvesting time and the processing of the honey. Hence, the small range provides information about the good quality of the honeys. In general the water content of eatable honey should not go beyond 20% (20).

Overall, the water content in Table 1 was similar in all the honey samples. The lowest of all samples was at 14.6% and the highest (by far) at 18.6% of GE04.

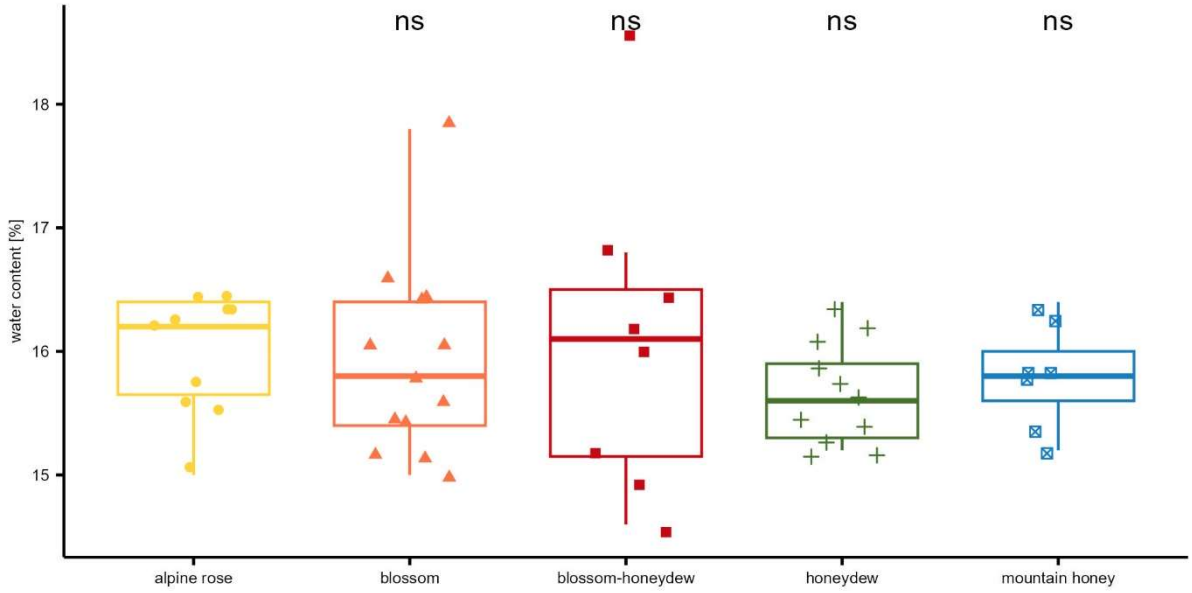

Figure 3: Boxplot of water content of the different honey types compared to the alpine rose samples

The range of the water content was not dependent on the honey variety but on its quality. Therefore, all the samples were of good quality and there was no significant difference between all of the samples because they were all in the same range. Striking was that the water content in the blossom honeys was by far the highest.

3.1.4. Electrical conductivity

Conductivity is the ability of a conductive substance to conduct or transfer energy or particles in a space. A substance's ability to conduct energy like heat or electricity is in some cases similar. Hence substances with high conductibility are metals and those with low abilities are gases. In honeys there usually is a positive correlation between mineral content, colour and electrical conductivity. The electrical conductivity is related to the ash content (mineral content) and acidity, giving insight into ions, organic acids and proteins. Therefore, honeys with higher conductibility are the ones with higher mineral content and acidity (5).

The conductivity of the blossom honeys ranges from 188 to 1058 µS/cm. The honey sample to stand out is sample BL13 with an electrical conductivity of 1058 µS/cm. If BL13 was excluded, the next higher value is 534 µS/cm which is appropriate to be declared as a blossom honey. Also 188 µS/cm is the lowest value of all honey samples which shows that blossom honeys tend to have lower mineral content and acidity.

The honeydew samples range from 813 to 1387 µS/cm which classifies them all as honeydew honeys.

The alpine rose honeys range from 205 to 782 µS/cm. This is possible, since they are likely to be a mixture of blossom and honeydew.

The mountain honeys range from 215 to 1306 µS/cm.

The blossom-honeydew honeys range from 388 to 990 µS/cm. The samples WB01 and WB02 both exceeded 800 µS/cm.

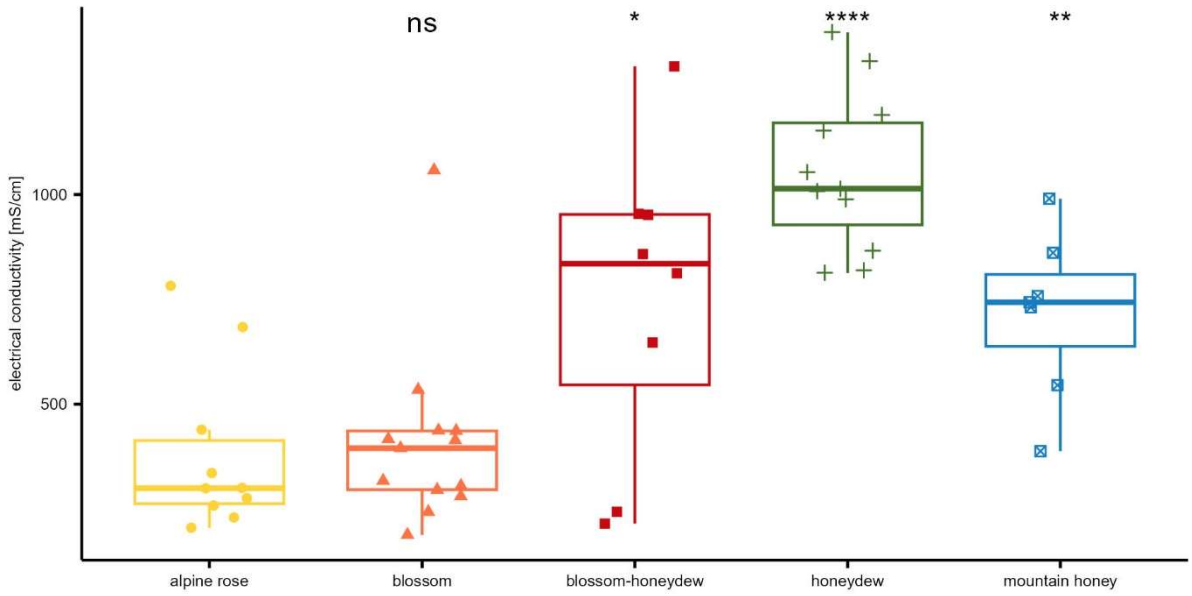

Figure 4: Boxplot of the electrical conductivity of the different honey types compared to the alpine rose samples

Resembling the pH-values, there was no significant difference between alpine rose honeys and blossom honeys and only a slight significant difference between the blossom-honeydew samples.

Furthermore, there was a significant difference between mountain honey and alpine rose honey.

Also, there was a highly significant difference between the alpine rose honeys and the honeydew honeys. The alpine rose samples did generally have a very low electrical conductivity, even lower than the blossom honeys, with just a few outliers.

3.2. Discriminant analysis

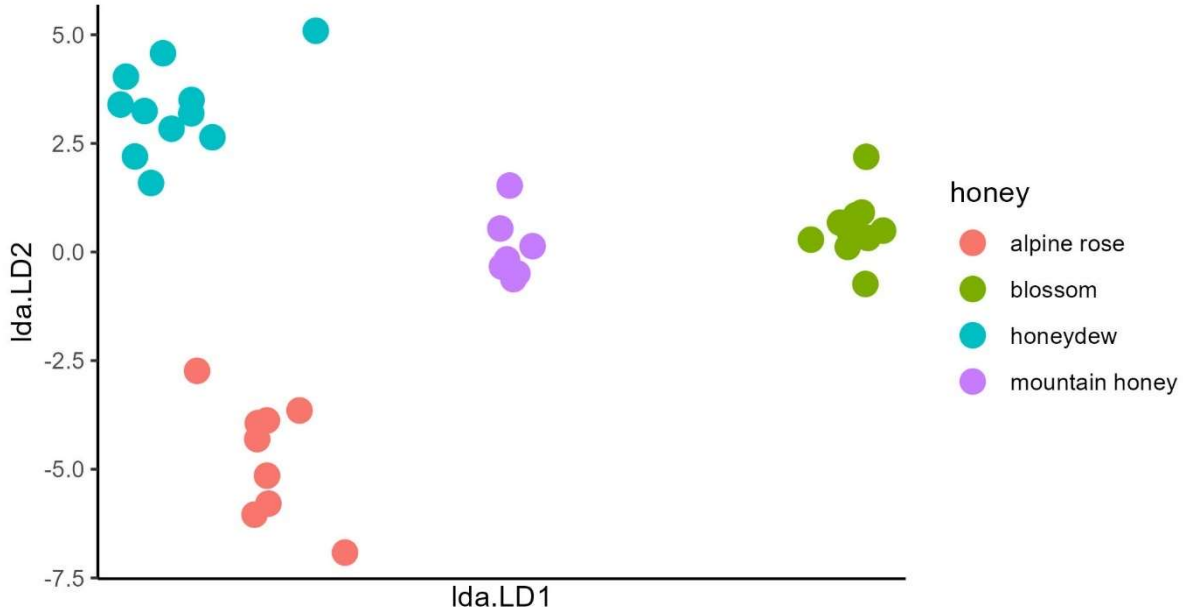

Figure 5: Discriminant analysis comparing the different honey types

The results in Figure 5 show that the different honey groups can be distinguished from each other. There is a clear difference between all of the samples assigned to a group of honey.

3.3. Essential oil composition

Table 2: essential oil composition of honey types without wrongly labelled honeys

ID	compound name	RI	alpine rose			RI	blossom			RI	mountain			RI	honeydew		
C01	3,3,5-trimethyl-heptane	907	0.11	±	0.06	908	0.26	±	0.49	907	0.23	±	0.06	908	0.37	±	0.15
C02	benzaldehyde	962	2.04	±	1.60	962	0.47	±	0.72	961	1.40	±	0.66	964	1.12	±	0.78
C03	dimethyl trisulfide	973	0.09	±	0.07	970	0.61	±	0.97	969	0.05	±	0.04	972	0.02	±	0.03
C04	2-pentyl-furan	991	0.18	±	0.12	992	0.10	±	0.11	992	0.06	±	0.03	992	0.01	±	0.02
C05	α-phellandrene	1004	0.06	±	0.06	1005	0.12	±	0.14	1104	0.21	±	0.22	1006	0.22	±	0.14
C06	1,2,3-trimethyl-benzene	1015	0.24	±	0.31	1015	0.21	±	0.28	1013	0.53	±	0.61	1015	0.43	±	0.44
C07	*p*-cymene	1026	0.14	±	0.11	1025	0.09	±	0.19	1024	0.14	±	0.17	1026	0.17	±	0.28
C08	benzeneacetaldehyde	1044	2.87	±	2.52	1087	3.15	±	5.90	1043	4.12	±	1.33	1046	4.04	±	2.29
C09	*cis* linalool oxide	1075	2.94	±	1.87	1075	0.70	±	1.01	1074	1.50	±	0.86	1071	1.24	±	1.06
C10	2-ethenyl-1,4-dimethyl-benzene	1091	1.94	±	1.27	1092	1.09	±	2.09	1091	1.44	±	1.29	1094	1.42	±	1.48
C11	hotrienol	1109	5.27	±	3.43	1110	5.80	±	7.60	1109	9.19	±	9.87	1110	8.12	±	7.44
C12	t-carenol	1137	0.26	±	0.17	1136	0.32	±	0.27	1137	0.37	±	0.49	1137	0.13	±	0.10
C13	lilac aldehyde B	1154	0.69	±	0.47	1155	0.08	±	0.12	1156	0.67	±	1.01	1153	0.21	±	0.16
C14	nerol oxide	1159	0.38	±	0.18	1163	0.21	±	0.31	1159	0.52	±	0.62	1160	0.32	±	0.25
C15	borneol	1171	0.29	±	0.21	1172	0.12	±	0.15	1169	0.47	±	0.26	1172	1.24	±	0.86
C16	α-terpineol	1193	0.80	±	0.57	1194	0.29	±	0.51	1192	0.74	±	0.54	1196	0.67	±	0.70
C17	decanal	1208	2.94	±	2.09	1210	0.39	±	0.78	1208	0.68	±	0.78	1212	0.11	±	0.05

C18	carvomenthenal	1225	3.65	±	2.44	1223	0.38	±	0.65	1222	0.74	±	0.81	1225	0.16	±	0.08
C19	1-(2-hydroxy-5-methylphenyl)-ethanone	1317	0.28	±	0.67	1317	0.05	±	0.07	1314	0.80	±	1.36	1317	0.16	±	0.14
C20	α-eudesmol	1649	0.87	±	0.99	1649	1.11	±	0.88	1646	0.17	±	0.09	1650	0.25	±	0.13
C21	myristic acid	1776	0.49	±	0.97	1779	2.22	±	2.50	1771	0.51	±	0.75	1777	0.17	±	0.38
C22	6,10,14-trimethyl-2-pentadecanone	1853	0.15	±	0.10	1852	0.47	±	0.19	1850	0.16	±	0.10	1854	0.20	±	0.13
C23	nonadecane	1906	0.84	±	0.24	1905	0.75	±	0.23	1903	0.94	±	0.41	1907	1.09	±	0.25
C24	palmitic acid	1977	11.54	±	6.50	1981	11.92	±	4.77	1977	13.16	±	4.43	1977	11.37	±	5.17
C25	hexadecanoic acid, ethyl ester	2001	0.45	±	0.63	2000	0.19	±	0.13	1997	0.21	±	0.21	2000	0.35	±	0.21
C26	9-eicosyne	2029	0.26	±	0.27	2027	2.18	±	2.67	2027	0.12	±	0.19	2028	0.26	±	0.68
C27	heneicosane	2107	4.09	±	0.74	2108	3.28	±	1.67	2105	8.51	±	5.39	2107	7.07	±	3.04
C28	*cis*-6-octadecanoic acid	2174	4.10	±	1.82	2176	5.33	±	2.93	2175	5.28	±	1.51	2170	5.59	±	1.23
C29	1-eicosanol	2282	1.62	±	0.32	2282	1.46	±	0.43	2282	1.47	±	0.62	2282	1.84	±	0.70
C30	tricosane	2310	17.12	±	2.44	2313	17.57	±	3.54	2308	18.01	±	6.93	2310	19.24	±	5.14
C31	tetracosane	2405	0.73	±	0.24	2406	1.08	±	0.35	2402	0.86	±	0.56	2405	0.65	±	0.19
C32	tricosyl pentafluoropropionate	2482	1.86	±	0.28	2483	1.58	±	0.40	2480	1.77	±	0.46	2483	2.02	±	0.61
C33	pentacosane	2508	13.26	±	2.68	2511	14.96	±	3.23	2507	11.83	±	2.88	2509	13.51	±	2.53
C34	heptacos-1-ene	2688	0.59	±	0.10	2687	0.61	±	0.26	2684	0.72	±	0.27	2685	0.84	±	0.42

C35	heptacosane	2709	10.09	±	2.03	2712	9.59	±	2.49	2706	7.27	±	3.64	2709	9.73	±	3.00
C36	13-methylheptacosane	2736	0.91	±	0.16	2736	0.70	±	0.38	2736	0.49	±	0.25	2740	0.77	±	0.36
C37	carbonic acid, decyl pentadecyl ester	2806	0.13	±	0.24	2805	0.05	±	0.05	2805	0.14	±	0.24	2806	0.06	±	0.09
C38	nonacos-1-ene	2889	0.33	±	0.08	2888	0.15	±	0.12	2883	0.29	±	0.06	2887	0.31	±	0.14
C39	nonacosane	2904	2.15	±	0.41	2906	1.12	±	1.00	2904	1.51	±	0.75	2907	1.90	±	0.72
C40	carbonic acid, decyl hexadecyl ester	2911	0.22	±	0.06	2913	1.23	±	1.15	2911	0.10	±	0.06	2910	0.13	±	0.07
C41	butyl hexacosyl ether	3079	0.52	±	0.13	3080	0.33	±	0.14	3080	0.35	±	0.12	3079	0.40	±	0.18
C42	ethyl triacontyl ether	3280	0.53	±	0.21	3279	0.50	±	0.52	3277	0.34	±	0.13	3282	0.41	±	0.17

3.3.1. Aroma profiles

For the aroma profiles, mislabelled honeys and blossom-honeydew samples were excluded.

Out of the given compounds, some could be identified as essential oil compounds such as α-phellandrene, *p*-cymene, *cis*-linaool oxide, hotrienol, t-carenol, nerol oxide, borneol, α-terpineol, carvomenthenal, α-eudesmol and 6,10,14-trimethyl-2-pentadecanone which are part of the monoterpenoids and sesquiterterpenoids.

There are also a few compounds which could be identified as hydrocarbons including saturated and unsaturated, for instance 3,3,5-trimethyl-heptane, 2-ethenyl-1,4-dimethyl-benzene, 1-(2-hydroxy-5-methylphenyl)-ethanone, nonadecane, 9-eicosyne, heneicosane, tricosane, tetracosane, tricosyl pentafluoropropionate, pentacosane, heptacos-1-ene, heptacosane, 13-methylheptacosane, nonacos-1-ene and nonacosane.

Benzaldehyde, benzeneacetaldehyde, lilac aldehyde B and decanal are part of the aldehydes and are also common in many types of honeys.

Also present are saturated and unsaturated fatty acids such as myristic acid, palmitic acid and *cis*-6-octadecanoic acid.

Esters of fatty acid were also identified. These include hexadecanoic acid ethyl ester, carbonic acid decyl pentadecyl ester and carbonic acid decyl hexadecyl ester.

The few other compounds that were found, such as dimethyl trisulfide, 2-pentyl-furan, 1-eicosanol, butyl hexacosyl ether and ethyl triacontyl ether are either trisulfides, furans, trimethylbenzenes or ethers.

The substances elevated in all of the samples are benzeneacetaldehyde, hotrienol, palmitic acid, *cis*-6-octadecanoic acid, tricosane, pentacosane, heptacosane and nonacosane.

In all of the honey samples these 42 compounds were identified. Since the substances occurred in all of the samples, a distinction between the samples has to be drawn from the differences in proportion of ingredients and their quality parameters.

3.3.2. Alpine rose honey

Analysing the compound composition in the alpine rose samples four compounds are found which can be distinguished from the other honey samples. These include 2-pentyl-furan, *cis*-linalool oxide, decanal and carvomenthenal.

3.3.3. Blossom honey

In the blossom honey group, five compounds are significantly different than other compounds. These include α-eudesmol, 6,10,14-trimethyl-2-pentadecanone, 9-eicosyne, nonacos-1-ene and carbonic acid decyl hexadecyl ester.

3.3.4. Honeydew

Noticeable for the honeydew group, borneol is higher than in the others. It differed significantly from the other samples and can be labelled as a marker compound for honeydew honeys. The substance is commonly occurring in essential oils from different plants and also in *Pinus sylvestris*, colloquially known as pine tree. Additionally it is also occurring in thyme (23).

3.3.5. Mountain honey

The only compound elevated in the mountain honey group was heneicosane. It differed significantly from the alpine rose honey group and the blossom honey group. Heneicosane belongs to the aliphatic alkane group which most of the aromas of citrus honeys belong to in, additionally to the linalool derivatives (23).

Other than that, the honey samples are all a mixture off likely floral honey and honeydew. One sample could clearly be identified as honeydew due to its quality parameters (pH and electrical conductivity), whereas the others perfectly displayed the mixture of the two groups.

4. Discussion

The quality parameters give a first insight into the classification of the honeys. Some samples differ from standard regulations and have to be reclassified or excluded.
To differentiate the alpine rose samples from the other honeys the essential oil compounds give more precise insight.

4.1. Comparison of the quality parameters

Starting with the pH-value the floral honeys in general have lower values than the honeydew honeys. Whereas the mean pH of the honeydew honeys is the highest. The alpine rose honey samples are even lower than the blossom honeys which indicates that all these honey samples are from mostly floral origin and/or with a small or no amount of honeydew. Unlike the alpine rose honey samples, the pH-values of the mountain honey samples are higher indicating the presence of honeydew honey in the mixture. The blossom-honeydew group, on the other hand, is a tad higher in average than the blossom honeys and only a bit lower in average than the honeydew honeys. These results reflect the mixture of blossom and honeydew honey with some samples having more floral amounts than others. There is a positive correlation between pH and electrical conductivity. Honeys with a higher pH-level tend to also have a higher electrical conductivity (9). In this case the correlation coefficient is at 0,9. The pH is decisive for the antimicrobial activities, it prohibits the growth of microorganisms (21).

Considering that the alpine rose samples are a mixture of blossom and honeydew honeys, it is remarkable that the free acidity exceeds the ones from the pure samples. Whereas by far the highest value stands out in the mountain honey group. It is possible, that this sample has honeydew dominates, but it could also be that this sample might have deteriorated. The blossom-honeydew samples are more or less consistently spread which indicates that they are an evenly mixture of blossom and honeydew.

Given the narrow range of the water content, it can be concluded, that the samples used are of good quality.

One honey sample of the blossom honey group (BL13) had an electrical conductivity of 1058 μS/cm. However, honeys above 800 μS/cm are classified as honeydew honeys. The mountain honey samples, just like the blossom-honeydew honeys, are also likely to be mixtures of blossom honey and honeydew honeys, but the highest and lowest value of electrical

conductivity stand out as either one of them. Considering the significant difference between the alpine rose honeys and the mountain honeys in electrical conductivity, this is probably because there are some samples in the mountain honeys which should actually be classified as honeydew honeys. Furthermore, the two samples WB01 and WB02 (blossom-honeydew) exceeded 800 μS/cm which most likely makes them pure honeydew honeys rather than blossom-honeydew honeys.

4.1.1. Deviations in Classification

The floral honey sample BL13 has a very high pH-value because of the fact that it is labelled as blossom honey. Also, the other quality parameters such as free acidity and electrical conductivity are too high so that this honey could be considered as a blossom honey. Based on the deviation in these parameters, this sample can be classified as honeydew honey.

The alpine rose samples AL01 and AL02 have very low electrical conductivity with 205 and 230 mS/cm. This could indicate that these samples are not a mixture of blossom and honeydew honeys but pure blossom honeys. The pH and likewise the free acidity of these two samples are lower than the values expected of blossom and honeydew mixtures. Also, none of the alpine rose samples have an electrical conductivity reaching 800 mS/cm which indicates, that there is not an overwhelming amount of honeydew in these samples.

The honey sample GE04 which had a very high free acidity value, also has the highest water content which makes it more unstable for fermentation than other honeys. This might indicate that the sample has turned bad.

The samples GE07 with an electrical conductivity of 215 μS/cm may indicate that its origin is from floral nectar and not honeydew. This matches with the pH and free acidity which share similarities with blossom honeys.

On the other hand, GE08 has an electrical conductivity of 1306 μS/cm. This high conductivity classifies these samples rather as pure honeydew and not mountain honey.

The honey samples WB01 and WB02 both have an electrical conductivity higher than 800 μS/cm which makes them both rather pure honeydew honeys than a mixture of honeydew and

blossom. The same is true for the samples WB06 and WB07. Both have very low electrical conductivity and lower pH. Given the low electrical conductivity, these samples are more likely pure blossom honeys.

Based on identifying the quality parameters, there are a few honey samples which stand out. This already shows that some of these samples are falsely classified. Therefore, these honey samples were excluded from the aroma profile analysis, due to the fact that they could tamper with the results.

4.2. Comparing the volatile compounds

Comparing the compounds to (24 Paper submitted), where the same honeys were analysed by an Solid phase microextraction (SPME) method instead of distillation, different compounds stand out and others are not present. Some compounds are from the same substance group, but different representatives such as α-phellandrene instead of β-phellandrene and *cis*-linalool oxide instead of linalool occur. Other compounds such as 1,4-dimethyl-δ-3-tetrahydroacetophenone, lilac aldehyde A and pinocarvone did not occur when using distillation.

The compounds that were identified as marker compounds for alpine rose honey using SPME are not the same as the ones identified through hydro distillation.
However, both extraction methods identified compounds to distinguish the honey samples from each other although more marker compounds were identified using the SPME method (24).

Benzaldehyde and benzeneacetaldehyde are very common compounds in honeys (1,25).

Hotrienol in particular is a labile compound among the terpenes. It can occur in non-thermally treated honey, but it is also thermally generated. If naturally present, the amount is lower in unripe honeys, suggesting that it develops through the ripening process of the honey (14). Since distillation was used to extract the compounds and the amounts of hotrienol in the samples are rather high, it is more likely, that it is a thermal artefact.

Palmitic acid is found in *Citrus*, *Astragalus* plants and honey thereof as well as in *Medicago* honeys (23).

Cis-6-octadecanoic acid, tricosane, pentacosane, heptacosane and nonacosane are most likely a chemical distinctive feature of the bees to recognize colony members. These so called cuticular hydrocarbons (CHCs) act as communication pheromones inside the beehive. These CHCs are probably a composition from nest building materials, the queen and diet. The CHC profile also changes with the age of the bee whereby forager bees possess a CHC profile which can be distinguished from others (26). Nurse bees tend to have the highest number of epicuticular profile compounds. The pattern changes over the bee's lifespan which is mostly because of the different tasks a bee executes (27). Cis-6-Octadecanoic acid was also found in the autumn honey of multifloral samples in Iraq but not in the samples of the spring honeys (28).

4.2.1. Alpine rose honey

2-pentyl-furan has a fruity odour and occurs in a variety of different foods, such as asparagus, potatoes, fruit juices, beverages and soybean products. However, it is also a biomarker for *Aspergillus fumigatus* (opportunistic pathogenic fungus). It can also occur in the thermal degradation of foods, which in this case could be due to the distillation process (29–32).

Cis-linaool oxide is a monoterpene derived from the plant. It is a common compound found in many honeys. Studies also show that lilac aldehydes are formed from linalool. lilac aldehyde B was only slightly elevated in the alpine rose group followed by the mountain honey samples, which also is common in *Rhododendron luteum*. *Cis*-linalool oxide has been found in various honey types such as Acacia, Caraway, Rosemary, Heather, Willow, Lavender, Citrus, Mint and Sage (1,14,23,25,33). This compound significantly distinguished alpine rose honey from blossom and honeydew honey.

Decanal, which also differed significantly from all of the other honey samples, is a common compound occurring in chestnut and heather honeys (34).

Carvomenthenal belongs to the group of p-Menth-1-ene-9-al which are menthane monoterpenoids. It is also a compound which distinguishes alpine rose honey highly significantly from all of the other samples. This compound is also derived from (*E*)-8-hydroxylinalool and via allylic rearrangement as well as cyclisation of 8-hydroxygeraniol. Nonetheless, the appearance in honey is limited (14,35).

4.2.2. Blossom honey

The compound α-eudesmol is a natural product found in many types of honeys such as multifloral honeys but also in Chestnut, Orange and various *Rhododendron* genus such as *Rhododendron calostrotum, Rhododendron lepidotum* and other organisms (36,37). This compound distinguishes blossom honey from mountain and honeydew honey.

6,10,14 trimethyl-2-pentadecanone is a compound found in different species of *Ficus*, it is known as a marker compound in Nigerian *Ficus* species but also in *Ficus carica* (edible fig). Also, it can be found in the essential oils of linden trees (38,39). This compound also distinguishes blossom honey highly significantly from all the other honey samples.

9-eicosyne falls under the group of terminal alkynes. Not much literature on 9-eicosyne can be found, but it evident is found in some seeds such as *Nigella sativa* (40,41). The same holds up for carbonic acid decyl hexadecyl ester.

Nonacos-1-ene is an alcohol which can be found in products derived from the fermentation of sugar in plants (42). It can also be found as a pheromone used for communication in insects such as *Lethocerus incdicus* (43).

4.2.3. Mountain honey

This kind of honey is not further defined in the “Honigverordnung” (20). However, in a decree it was settled that mountains are spatially closed, higher parts of the earth’s surface that stand out clearly from the lower level surroundings. Most common is the differentiation based on the relative altitude difference in highlands and uplands. The honey gained from the highlands has to contain pollen of *Ericacae* which only grows above the treeline, whereas honey from the uplands can be a mixture of floral honey and honeydew (contains *Pinaceae* pollen) (44). The honey samples were only labelled as “Gebirgshonig” and, therefore it was not classified if they are from the uplands or the highlands.

4.3. Is it possible to distinguish Alpine rose honey from other honeys

There are four substances which make it possible to distinguish alpine rose honey from the other honey samples. The compound *cis*-linalool oxide makes it possible to distinguish alpine

rose honey significantly from blossom honey and honeydew honey. However, this compound does not distinguish it from the mountain honey samples.

2-pentyl-furan, on the other hand is distinguishing the alpine rose samples significantly from all of the other samples.

Decanal and carvomenthenal also distinguish alpine rose honey from all other samples even highly significantly. Therefore, these two compounds can be defined as marker compounds for alpine rose honey.

4.3.1. Differentiation based on quality parameters

Moreover, the quality parameters listed in Table 1 give insight into the distinction between the honey samples. Considering the pH and the electrical conductivity, a clear distinction becomes apparent. It shows that alpine rose honeys can be classified as blossom honeys with nectar from the *Rhododendron* species.

4.4. Further differentiation options

Besides the quality parameters and the aroma profiles, there would have been another option for further differentiation of the alpine rose honey. Melissopalinology or pollen analysis is often used to distinguish honeys from each other. Every honey contains pollen grains which give good insight about the fingerprint of the environment since the grains are mainly from the plants or honeydew elements that the bees gathered. To differentiate the pollen, the samples have to be centrifuged first and then be looked at under the microscope (45).
This was not applied with the samples used here. It has to be considered that it could have given some more insight into the honey samples and maybe could have helped to further differentiate them.

Melissopalinology, however, requires experienced analysts, and besides being very time-consuming, the outcome depends on the expert's ability and judgement (8,15). The pollen of *Rhododendron ferrugineum* and *Rhododendron hirsutum* are established and could be identified, which could give further insight if continued to be evaluated (46).

4.5. Problematic because of lack of samples or amounts

Forty-nine honey samples were selected but after analysing the quality parameters, a few of them had to be excluded due to incorrect classification.

No monofloral honeys were used, and all of the products were labelled as mixed or, if they were, just as honeydew honeys. The amount of honey in these samples varied from 250-500g. However, some amount was taken for the quality parameters. Therefore, the amount of honey decreased slightly.

The distillation process required 200g of honey which is a great amount. Also, the distillation process probably would have been a bit more precise if there were more honey on hand. Sadly, some of the suppliers only had these amounts available, so the least amount possible had to be used. Besides that, if monofloral samples had been used, some floral indicators would have been more prominent, and it would have been easier to match the floral markers with its origin. The markers identified for the blossom honey samples can, therefore, not be used to match it to a specific type of nectar but as markers for floral origin.

4.6. How accurate is Distillation

Distillation is performed under heat exposure. This has an effect on the outcome since the sugars and amino acids react with it. Thermal artefacts tend to occur, and sensitive compounds are likely oxidized or decomposed whereas new ones emerge (15). Even though some substances are normally present in the aroma of honeys, some like e.g. hotrienol tend to favour from the higher temperatures used for the extraction by distillation (18). Since oxygenated volatile organic compounds can be water soluble but essential oils are not, some organic compounds could go missing through the distillation process. Moreover, heat should not be applied to honey since it promotes the occurrence of thermal artefacts. Therefore, using hydro distillation to extract essential oils from honey might not be the most efficient method (14).

Studies have proven that SPME and ultrasonic solvent extraction (USE) are more accurate and fewer associated with the emergence of thermal artefacts (15).

However, it was possible to extract compounds which can be described as marker compounds, to distinguish alpine rose honey from other samples. However, 2-pentyl-furan which was identified as an alpine rose honey marker and the compound hotrienol which was commonly

found in all of the honey samples, could be thermal artefacts emerged through the distillation process.

5. Conclusion

Overall, four compounds could be identified that quantitatively distinguish alpine rose honey from other honey samples.

The compound 2-pentyl-furan differentiates alpine rose honey from mountain honey and blossom honey. It is also a compound which distinguishes honeydew honey from blossom honey. This compound could indicate that there is some alpine rose nectar in a few honeydew samples.

Cis-linalool oxide is a compound which distinguishes alpine rose honey from blossom and honeydew honeys. However, it does not differentiate it from the mountain honey samples which could be due to the fact, that the mountain honeys are gathered from partly the same areas and have some things in common.

Decanal and carvomenthenal are the two compounds which distinguish the alpine rose samples highly from all the other samples. Therefore, these compounds could be addressed as marker compounds for alpine rose honeys.

There is one compound which could be found in the *Rhododendron* genus but also in various types of multifloral honeys. The compound is α-eudesmol, which is a compound that distinguishes blossom honey from honeydew and mountain honeys. However, it does not differentiate the blossom honey samples from the alpine honey samples. This suggests that the compound is also appearing in the alpine rose samples but not in such quantities as it occurs in the blossom honeys.

As for other volatile markers, e.g. hotrienol, which is often found in citrus honeys, is also found in all of the honey samples in different quantities. It appears to be a volatile marker but it could also be an indication for thermal artefacts which occur during the distillation process (8,18).

Besides using mixtures of various honeys and no monofloral honeys, the identification of honey volatiles is dependent on the extradition method with other extraction methods having little to almost no artefacts (15,16,25).

6. Table directory

Table 1 7
Table 2 16

7. List of Illustrations

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8. Bibliography

1. Machado AM, Miguel MG, Vilas-Boas M, Figueiredo AC. Honey Volatiles as a Fingerprint for Botanical Origin—A Review on their Occurrence on Monofloral Honeys. Molecules. 2020 Jan;25(2):374.

2. Pita-Calvo C, Vázquez M. Differences between honeydew and blossom honeys: A review. Trends Food Sci Technol. 2017 Jan 1;59:79–87.

3. Lipp J, Böttcher FK, Zander E, Koch A, Vorwohl G, Lipp G. Der Honig. 3. Stuttgart: E.U. Verlag Eugen Ulmer; 1994. 205 p.

4. Baroni MV, Nores ML, Díaz MDP, Chiabrando GA, Fassano JP, Costa C, et al. Determination of Volatile Organic Compound Patterns Characteristic of Five Unifloral Honey by Solid-Phase Microextraction−Gas Chromatography−Mass Spectrometry Coupled to Chemometrics. J Agric Food Chem. 2006 Sep 1;54(19):7235–41.

5. da Silva PM, Gauche C, Gonzaga LV, Costa ACO, Fett R. Honey: Chemical composition, stability and authenticity. Food Chem. 2016 Apr 1;196:309–23.

6. Mizrahi A, Lensky Y. Bee Products, Properties, Applications and Apitherapy. Plenum Press; 1996.

7. Bogdanov S, Martin P. Honey Autheticity: a Review.

8. Cuevas-Glory LF, Pino JA, Santiago LS, Sauri-Duch E. A review of volatile analytical methods for determining the botanical origin of honey. Food Chem. 2007 Jan 1;103(3):1032–43.

9. Seraglio SKT, Silva B, Bergamo G, Brugnerotto P, Gonzaga LV, Fett R, et al. An overview of physicochemical characteristics and health-promoting properties of honeydew honey. Food Res Int. 2019 May 1;119:44–66.

10. Popescu R. The genus Rhododendron_ An ethnopharmacological and toxicological review. J Ethnopharmacol. 2013;

11. Oddo LP, Piazza M, Sabatini A, Accorti M. Characterization of unifloral honeys. 1995.

12. Zimmermann E. Aromatherapie für Pflege- und Heilberufe. München: Johannes Sonntag Verlagsbuchhandlung GmbH; 1998. 252 p.

13. Dingermann T, Hiller K, Schneider G, Zündorf I. Schneider Arzneidrogen. 5th ed. München: Elsevier; 2004. 627 p.

14. Jerković I, Kuś PM. Terpenes in honey: occurrence, origin and their role as chemical biomarkers. RSC Adv. 2014 Jul 21;4(60):31710–28.

15. Jerković I, Mastelic J, Marijanović Z, Klein Z, Jelic M. Comparison of hydrodistillation and ultrasonic solvent extraction for the isolation of volatile compounds from two unifloral honeys of Robinia pseudoacacia L. and Castanea sativa L. - ScienceDirect [Internet]. [cited 2023 Sep 2]. Available from: https://www.sciencedirect.com/science/article/pii/S135041770700003X

16. Castro-Vázquez L, Pérez-Coello MS, Cabezudo MD. Analysis of volatile compounds of rosemary honey. Comparison of different extraction techniques. Chromatographia. 2003 Feb;57(3–4):227–33.

17. Manyi-Loh CE, Ndip RN, Clarke AM. Volatile Compounds in Honey: A Review on Their Involvement in Aroma, Botanical Origin Determination and Potential Biomedical Activities. Int J Mol Sci. 2011 Dec;12(12):9514–32.

18. Alissandrakis E, Tarantilis PA, Harizanis PC, Polissiou M. Evaluation of four isolation techniques for honey aroma compounds. J Sci Food Agric. 2005;85(1):91–7.

19. Yadata D. Detection of the Electrical Conductivity and Acidity of Honey from Different Areas of Tepi. Food Sci Technol. 2014;

20. Honigverordnung [Internet]. [cited 2023 Dec 18]. Available from: https://www.ris.bka.gv.at/Dokumente/BgblAuth/BGBLA_2004_II_40/BGBLA_2004_II_40.html

21. Pascual-Maté A, Osés SM, Fernández-Muiño MA, Sancho MT. Methods of analysis of honey. J Apic Res. 2018 Jan 1;57(1):38–74.

22. Singh I, Singh S. Honey moisture reduction and its quality. J Food Sci Technol. 2018 Oct;55(10):3861–71.

23. Tofighi Z, Es-haghi A, Asl MM, Tajic AR, Navai MS, Tavakoli S, et al. Investigation of chemical keys for relationship between plants and their unifloral honeys by hydrodistillation and SPME and biological activities of honeys. Eur Food Res Technol. 2014 Apr 1;238(4):665–73.

24. Novak J. Volatiles Are Distinguishing Rhododendron Honey ('Almrauschhonig') from Other Austrian Honeys).

25. Kaškonienė V, Venskutonis PR, Čeksterytė V. Composition of volatile compounds of honey of various floral origin and beebread collected in Lithuania. Food Chem. 2008 Dec 15;111(4):988–97.

26. Vernier CL, Krupp JJ, Marcus K, Hefetz A, Levine JD, Ben-Shahar Y. The cuticular hydrocarbon profiles of honey bee workers develop via a socially-modulated innate process. Scott K, Dulac C, editors. eLife. 2019 Feb 5;8:e41855.

27. Naccarato A, Cavaliere F, Tassone A, Brandmayr P, Tagarelli A, Pirrone N, et al. In vivo solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) assay to identify epicuticular profiles across task groups of Apis mellifera ligustica workers. J Entomol Acarol Res [Internet]. 2019 Apr 19 [cited 2024 Apr 28];51(1). Available from: https://www.pagepressjournals.org/jear/article/view/7874

28. Salman MA, Alhilfi DFA and AZA. Effect of Seasonal on Strength of Bee and Volatile Compounds in Multi Flora Honey. Ann Romanian Soc Cell Biol. 2021 Jun 11;25(6):10533–41.

29. Tabibpour M, Yamini Y, Ahmadi SH, Esrafili A, Salamat Q. Carbon fibers modified with polypyrrole for headspace solid phase microextraction of trace amounts of 2-pentyl furan from breath samples. J Chromatogr A. 2020 Jan 4;1609:460497.

30. Bhandari S, Chambers S, Pearson J, Syhre M, Epton M, Scott-Thomas A. Determining the limits and confounders for the 2-pentyl furan breath test by gas chromatography/mass spectrometry. J Chromatogr B. 2011 Sep 15;879(26):2815–20.

31. Hu G, Zhu Y, Hernandez M, Koutchma T, Shao S. An efficient method for the simultaneous determination of furan, 2-methylfuran and 2-pentylfuran in fruit juices by headspace solid phase microextraction and gas chromatography–flame ionisation detector. Food Chem. 2016 Feb 1;192:9–14.

32. 2-Pentylfuran | 3777-69-3 [Internet]. [cited 2024 Mar 16]. Available from: https://www.chemicalbook.com/ChemicalProductProperty_DE_CB8679998.htm

33. Kuś PM, Jerković I, Marijanović Z, Tuberoso CIG. Screening of Polish Fir Honeydew Honey Using GC/MS, HPLC-DAD, and Physical-Chemical Parameters: Benzene Derivatives and Terpenes as Chemical Markers. Chem Biodivers. 2017;14(9):e1700179.

34. Jerković I, Tuberoso CIG, Marijanović Z, Jelić M, Kasum A. Headspace, volatile and semi-volatile patterns of *Paliurus spina-christi* unifloral honey as markers of botanical origin. Food Chem. 2009 Jan 1;112(1):239–45.

35. Jerković I, Prđun S, Marijanović Z, Zekić M, Bubalo D, Svečnjak L, et al. Traceability of Satsuma Mandarin (Citrus unshiu Marc.) Honey through Nectar/Honey-Sac/Honey Pathways of the Headspace, Volatiles, and Semi-Volatiles: Chemical Markers. Molecules. 2016 Sep 29;21(10):1302.

36. PubChem. alpha-Eudesmol [Internet]. [cited 2024 Feb 13]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/92762

37. Vazquez L, Celeiro M, Sergazina M, Dagnac T, Llompart M. Optimization of a miniaturized solid-phase microextraction method followed by gas chromatography mass spectrometry for the determination of twenty four volatile and semivolatile compounds in honey from Galicia (NW Spain) and foreign countries. Sustain Chem Pharm. 2021 Jun 1;21:100451.

38. Adeyemi A, Oluigbo C, Esan AO, Bello M, Oladoye S, Emmanuel C, et al. Chemical Composition and Antimicrobial Activity of the Essential oils of 14 known Ficus species -A Concise Review. Biointerface Res Appl Chem. 2021 Dec 5;12:8003–34.

39. Essential Oil Composition of Tilia cordata Flowers [Internet]. [cited 2024 Mar 2]. Available from: https://www.tandfonline.com/doi/epdf/10.1080/0972060X.2017.1359681?needAccess=true

40. S D, Sridhar S, Palanisami E. Chemical composition and antioxidant activities of black seed oil (Nigella sativa L.). Int J Pharm Sci Res. 2016 Nov 1;7:4473–9.

41. Showing Compound 9-Icosyne (FDB020990) - FooDB [Internet]. [cited 2024 Feb 27]. Available from: https://foodb.ca/compounds/FDB020990

42. de Carvalho FM de A, Schneider JK, de Jesus CVF, de Andrade LN, Amaral RG, David JM, et al. Brazilian Red Propolis: Extracts Production, Physicochemical Characterization, and Cytotoxicity Profile for Antitumor Activity. Biomolecules. 2020 May;10(5):726.

43. Devi MR, Ummalyma SB, Brockmann A, Raina V, Rajashekar Y. Nutritional properties of giant water bug, Lethocerus indicus a traditional edible insect species of North-East India. Bioengineered. 2023 Dec 31;14(1):2252669.

44. RIS Dokument [Internet]. [cited 2024 Feb 14]. Available from: https://www.ris.bka.gv.at/Dokumente/Vwgh/JWT_1997100167_20000904X00/JWT_19971 00167_20000904X00.html

45. Corvucci F, Nobili L, Melucci D, Grillenzoni FV. The discrimination of honey origin using melissopalynology and Raman spectroscopy techniques coupled with multivariate analysis. Food Chem. 2015 Feb 15;169:297–304.

46. PalDat [Internet]. [cited 2024 Feb 16]. Available from: https://www.paldat.org/search/genus/Rhododendron