THESIS ON NATURAL AND EXACT SCIENCES B240

Application of Gas Chromatography-Olfactometry (GC-O) and Correlation with Sensory Analysis

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TTÜ PRESS

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This dissertation was accepted for the defence of the degree of Doctor of Philosophy (Chemical and Material Sciences) on October 04, 2017

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Defence of the thesis: November 24, 2017. Tallinn

Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Sirli Rosenvald

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ISSN 1406-4723
ISBN 978-9949-83-163-0 (publication)
ISBN 978-9949-83-164-7 (PDF)

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LOODUS- JA TÄPPISTEADUSED B240

Gaaskromatograaf-olfaktomeetri (GC-O) rakendusvõimalused ja korreleerimine sensoorse analüüsiga

SIRLI ROSENVALD

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LIST OF PUBLICATIONS

The following publications form the basis of this dissertation and are reproduced in the appendices with permission from the publishers. Publications I-III are published under the maiden name – Seisonen.

I Vene, K., **Seisonen, S**., Koppel, K., Leitner, E., Paalme, T. A Method for GC-Olfactometry Panel Training. Chemosensory Perception, 6 - 4, 179-189, (2013)

II **Seisonen, S**., Kivima, E., Vene, K. Characterization of the aroma profile of different honeys and corresponding flowers using SPME-GC/MS and GC-Olfactometry. Food Chemistry,169, 34–40, (2015)

III **Seisonen, S**., Vene, K., Koppel, K. The current practice in the application of chemometrics for correlation of sensory and gas chromatographic data. Food Chemistry, 210 (1), 530−540, (2016)

IV Kortesniemi, M., **Rosenvald, S**., Laaksonen, O., Vanag, A., Ollikka, T., Vene,K., Yang, B. Sensory and chemical profiles of Finnish honeys of different botanical origins and consumer preferences. Food Chemistry, *in press*, accepted manuscript.

SUMMARY OF AUTHOR`S CONTRIBUTION

I In Publication I, the author planned and performed the statistical analysis for monitoring the GC-O panel performance and interpreted the data.

II In Publication II, the author participated as a GC-O assessor, analysed the results, wrote the manuscript and is the corresponding author.

III In Publication III, the author carried out the literature review, wrote the manuscript and is the corresponding author.

IV In Publication IV, the author carried out the GC-O experiments and statistical analysis of correlating GC-O and sensory data, interpreted the result of GC-O experiments and correlation with sensory data and wrote a part of the manuscript.

LIST OF PRESENTATIONS

I Seisonen, S., Kivima, E., Vene, K. Characterization of the aroma profiles of different honeys and corresponding flowers using SPME-GC/MS and GC-Olfactometry. 9 th Baltic Conference on Food Science and Technology: Foodbalt 2014, May 8-9, 2014, Jelgava, Latvia (oral presentation).

II Seisonen, S., Kajava, K., Kuldjärv, R., Vene, K. Correlation of gas chromatography-olfactometry and sensory descriptive analysis of oregano samples by PLSR. 8th International Conference of Partial Least Square Regression and Related Methods, May 26-28, 2014, Paris, France (poster presentation).

III Seisonen, S. Current practice in correlating volatile composition with sensory analysis. VLAG course Advanced Food Analysis, January 26-30, 2015, Wageningen, The Netherlands (poster presentation).

IV Rosenvald, S., Vene, K., Koppel, K. An overview of statistical methods currently used for correlating sensory properties of food with its volatile composition. Sensometrics conference, July 26-29, 2016, Brighton, UK (oral presentation).

V Rosenvald, S. Correlation of GC-O and sensory data of Finnish honeys – overview of statistical methods. Functional materials and technologies" graduate school conference, March 7-8, 2017, Tartu, Estonia (oral presentation).

VI Rosenvald, S., Kortesniemi, M., Laaksonen, O., Ollikka, T., Vene, K., Yang, B. Correlation of gc-o and sensory data of Finnish honeys – methods and challenges. 12th Pangborn Sensory Science Symposium, August 20-24, 2017, Rhode Island, USA (poster presentation).

ACKNOWLEGMENTS

I would like to thank my supervisor Professor Toomas Paalme for the opportunity to start my research in CFFT, for his guidance and contribution to this thesis. My special thanks of gratitude go to my co-supervisor Kristel Vene who revealed to me the wonderful world of food aroma and have advised, encouraged and inspired me the hole journey.

I am very grateful to Andy Taylor and Mari Sandell for accepting to be the official opponents of my thesis. I thank Andy Taylor for useful advice and comments during pre-review of my thesis.

I wish to thank Evelin Kivima for giving me the possibility to be part of her honey study, Kadri Koppel for her help with reviewing the literature on chemometric techniques, and Maaria Kortesniemi and Oskar Laaksonen for fruitful co-operation while investigating Finnish honey samples.

I am also grateful to all of my colleagues from CFFT, especially Aleksei Kaleda for his everlasting willingness to help me with R software. I thank Rain Kuldjärv, Julia Rosend and Edgar Kelman whose advice and support have been most valuable.

I wish to thank all of my friends and relatives for supporting me but my endless gratitude goes to my husband Martin Rosenvald, and my parents Ülle and Valeeri Seisonen whose unconditional love and support have brought me here.

I wish to thank the institutions that have provided financial support for this work, including EU Regional Development Fund, projects EU29994 and EU48667, the Estonian Ministry of Education research grant IUT1927, as well as, SA Archimedes and doctoral schools in Faculty of Chemical and Materials Technology at TUT. This work has been partially supported by ASTRA “TUT Institutional Development Program for 2016-2022” Graduate School of Functional Materials and Technologies (2014-2020.4.01.16-0032).

I dedicate this thesis to my two beautiful children Riko and Keira who are the light of my life.

ACRONYMES

AEDA	aroma extract dilution analysis
AHC	agglomerative hierarchical clustering
ANOVA	analysis of variance
CA	correspondence analysis
CATA	check all that apply
CFFT	Centre of Food and Fermentation Technologies
CV	cross validation
DF	detection frequency
ESS	error sum of squares
FD	flavor dilution (factor)
FID	flame ionization detector
FMTM	fragrance materials test mix
FPD	flame photometric detection
GC	gas chromatography
GC-O	gas chromatography-olfactometry
HCA	hierarchical clustering analysis
MF	modified frequency
MS	mass spectrometry
NIF	nasal impact frequency
OAV	odor activity value
OSV	odor spectrum value
PCA	principal component analysis
PI	posterior intensity
PLS-DA	partial least square – discriminant analysis
PLSR	partial least square regression
QDA	quantitative descriptive analysis
RMSE	root mean square error
SDA	stepwise discriminant analysis
SNIF	surface of nasal impact frequency
TOF-MS	time-of-flight mass spectrometry
VIP	variable importance to projection

INTRODUCTION

Flavour is one of the most important characteristics of any food product. Its critical role in determining the way consumers assess the food quality has made it a key area of research and development in food industry. Nowadays flavour science has become a very broad subject aiming to provide a comprehensive understanding of flavour, from its generation in food, stability during storage, to its perception during eating (Voilley et al. 2006).

Very important part of food flavour is its aroma, which is the response of the olfactory epithelium in the roof of the nasal cavity to volatiles entering the nasal passage (Baigrie, 2003). In general, the aroma of a food consists of many odor-active volatile compounds, only a few of which are sensorially relevant. One of the major problems in aroma research is to select those compounds that significantly contribute to the aroma of a food (Blank, 2001).

The aroma of food is investigated by using sensory analysis and/or instrumental analysis of volatiles. Sensory analysis uses only human senses as instruments of measurement. Instrumental analysis involves separation of aroma compounds and recording of the mass detector's signal. Both methods have its *pros and cons* and therefore combining those would give the best results. This can be done running instrumental and sensory methods in parallel or hyphenating gas chromatography (GC) with mass spectrometry (MS)/flame ionization detector (FID) the olfactometry port for sniffing (sensory analysis). Thus, gas chromatography-olfactometry (GC–O) provides not only an instrumental, but also a sensorial analysis (Zellner et al., 2008). Although, GC-O uses human senses for detection in present work it is classified as instrumental method of analysis.

GC-O is a valuable method in food aroma analysis as very often aroma compounds are present in such low concentrations that even the most sensitive MS detectors are not capable to detect them. Therefore, GC-O is often a method providing the most accurate aroma profile of the food products. Unfortunately, all the bias coming from human fluctuation is observed similarly to sensory analysis and training of the GC-O assessors is as crucial as for sensory analysis to get reliable results. As brought out by Van Ruth et al. (2001b), the training of the GC-O assessors will reduce the noise level. Also, the data handing and typical mistakes are rather similar to classical sensory analysis. There are still some principal differences. For example, during the sensory analysis, assessors must evaluate different aroma attributes from a complex matrix, but GC-O analysis enables assessment of each separate aroma characteristic. From one side, it makes the evaluation process easier as the assessor does not have to deal with the effect of the whole product matrix. On the other hand, making the assessments without accounting for the matrix effects decreases the reliability of the results obtained. GC-O will not reflect the sensory perception with full accuracy. We cannot account for the interactions in mixtures of volatiles as the non-volatile part of the matrix has a great influence on how the volatiles are released. Though, when analysing samples by using headspace extraction methods, the intensities perceived with GC-

O from samples with different non-volatile fractions are well correlated with odor attribute intensities evaluated by using classical sensory analysis (Sáenz-Navajas et al., 2010). The retronasal aroma of the food product is less predictable by GC-O results as there is even bigger interactions between odor-active volatiles and different non-volatile compounds. Also, influences during eating that are coming from mixing with saliva and chewing process affect aroma release. From the technical side, when sniffing from GC-O port, assessors have only seconds to react and give all valuable information without a possibility to take a second sniff to re-evaluate.

The importance of GC-O in food aroma analysis should not be underestimated. Still, like any other technique it requires proper experiment design and task establishment, assessors suitable for selected methods and application of advanced data handling techniques, to take the maximum gain from the experiments. This work was initiated by the practical need to study numerous factors that influence the reliability and usefulness of the information gained by GC-O experiments to improve the methodology of GC-O analysis and the interpretation of the results.

This thesis covers different methodological aspects of using data from GC-O analysis, as well as set up of the experiments in terms of data collection techniques as the input for data analysis. The work covers evaluation of GC-O assessor`s performance as the indicator of the reliability of the raw data, different GC-O and statistical techniques to fulfil the aims of various experiments and gives a comprehensive overview of different aspects in techniques used for correlation of GC and sensory data, illustrated by a case study on Finnish honey samples.

2 LITERATURE REVIEW

2.1. Gas-chromatography – olfactometry (GC-O)

First application of GC-O dates to 1964 (Fuller et al., 1964) and since then it has been a useful technique for analysing odor-active compounds from different matrixes. GC-O is a term used to describe the analytical technique that uses human assessors to detect and evaluate volatile compounds eluting from a GC separation (Figure 1).

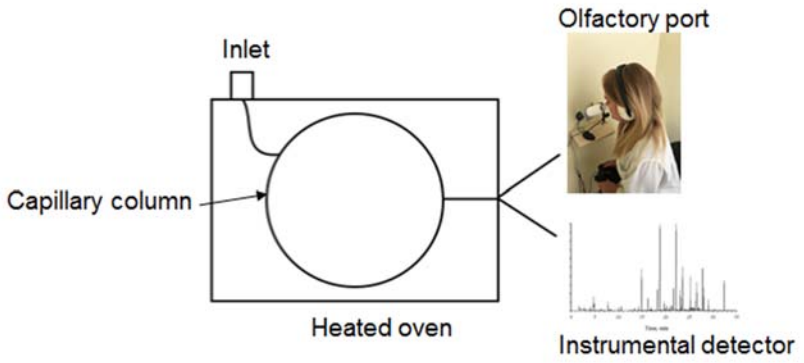

Figure 1. Scheme of a gas chromatograph coupled with olfactory port

GC-O is a valuable method for the identification of odor components from a complex mixture of volatile compounds (Brattoli et al., 2013). Because most instrumental detectors measure a mass-related signal, the peak profiles gained does not reflect the odor profile of the samples as all volatile compounds are recorded, even those without odor-activity. Besides, as the information about human perception is not provided, a linear correlation between a quantified substance and an olfactory stimulus cannot be made (Brattoli et al., 2013). Also, it is widely known that the chemical/physical detectors are often not as sensitive as human nose for detecting odor-active compounds from different samples (Acree et al., 1984). For example, Koutidou et al. 2017, managed to identify only eighteen aroma compounds in onion-tomato puree by using one-dimensional GC–MS, though, thirty-two compounds were detected with GC-O. To achieve enhanced separation and resolve co-eluting compounds, comprehensive two-dimensional gas chromatography, coupled to time-of-flight mass spectrometry (GC × GC–TOF MS) was used. Still, even with two-dimensional GC, 5 compounds detected with GC-O remained undetected with GC-MS.

GC-O was initially described as a screening method to determine whether a volatile compound found in a sample had odor activity or not. Nowadays, applications of the technique have become more advanced, and the method is also

used to assign a relative importance to each of the volatile compound identified as being odor-active (Delahunty et al., 2006).

Although humans have a good sensitivity to aroma compounds, there are multiple challenges that should be addressed. Firstly, there are very significant differences in olfactory ability between humans; odor thresholds can vary significantly among individuals and some people, with an otherwise normal sense of smell, are unable to detect families of similar aroma compounds. A recent comprehensive study on more than 1600 individuals revealed that specific anosmia is a highly common phenomenon (Croy et.al., 2015). The authors tested 20 odors with 200 participants for each odor and showed that the rate of specific anosmia to these odors varied from 0.5% to 20.4%. Statistical estimations yielded an estimated prevalence of 51.9% of specific anosmia to at least one of the 20 assessed odors. Also, the olfactory response of an individual is known to vary over time, even during a single day, and with the speed of breathing. Sensitivity may also fluctuate due to health status and mood (Brattoli et al., 2013). Therefore, selection of GC-O panellists and using sufficient number of panellists is of high importance.

Detection of the odor is possible when the concentration of the compound is above the odor threshold value. Odor threshold value is the minimum concentration of the compound which is enough for the recognition of the odor (Belitz et al., 2004). The perceived intensity of the odor could be characterized by the ratio between compound concentration and its odor threshold, which is called odor activity value (OAV). In case of each odor-active compound eluting from the GC column and having OAV larger than 1, every assessor has a potential to detect the odor, measure the duration of the odor-activity, describe the quality of the odor and to quantify the intensity of it. Based on this, various GC-O techniques have been developed (Delahunty et al., 2006). Firstly, there are dilution techniques which are based on diluting the odor-active compounds to their thresholds. Aroma extract dilution analysis (AEDA) and combined hedonic aroma response measurement (Charm Analysis) are the dilution methods that are used most often. AEDA was first presented by Ullrich et al. (1987) and it measures the highest sample dilution at which the odour of the analysed compound is still detectable for the assessor (flavor dilution factor). Charm analysis, proposed by Acree et al. (1984), also records the duration of odours which is considered with the final dilution at which the compound is detected. To compare the results of different studies using Charm analysis, Acree (1997) introduced odor spectrum value (OSV), which is odor potency determined with Charm Analysis normalized to the most potent odorant detected.

Detection frequency (DF) methods measure the intensity of the compound by calculating the number of assessors detecting the odor. Based on detection frequencies two factors could be calculated; the number of detections by the assessors – nasal impact frequency (NIF) or combining NIF value with the duration of the odor by each assessor – surface of nasal impact frequency (SNIF). NIF value is 0 when none of the assessors sensed the odor at given retention time, and it is 1 when all the assessors sensed the odor (Brattoli et al. 2013). NIF value can also be

expressed as a percentage and for SNIF value the percentage of the detection should be multiplied with the total duration of the odor.
Finally, posterior intensity (PI) methods measure the maximum intensity of perceived odor on previously determined scale once the compound has eluted (Delahunty et al. 2006). Modified frequency (MF) is a method proposed by Dravnieks (1985) and is combining frequency and intensity values.

$$MF = \sqrt{F(\%) \times I(\%)} \qquad (1)$$

where F(%) is the detection frequency of an aromatic attribute expressed as percentage of maximum frequency of the panel and I(%) is the average intensity expressed as the percentage of the maximum intensity of the panel. Detection frequency method proposes that the proportion of people able to detect the presence of a given odorant is related to its concentration or importance. This strategy has the doubtless advantage of its simplicity and requires little training from the judges (Ferreira et al., 2003)

All the above-mentioned methods are using different approaches but share the same goal – to measure the importance of different odor-active compounds. The quantification with GC-O is mainly not aimed to measure the differences in absolute concentrations of the compounds but rather to evaluate the impact each compound has on overall sensory properties (aroma) of the samples or to measure the relative concentrations of the compounds between the samples. GC-O detection frequency method have been used by Pollien et al. 1999 to create calibration curves for measuring the concentration of 1-octen-3-one in coffee. Calibration curves were composed from 3 and 4 concentration points and had determination coefficients in range of 0.82-0.99.

Posterior intensity methods are measuring the intensities of perceived compounds which should refer to the compound concentrations or importance. Ferreira et al. 2003 used a simple 3-point scale to build calibration graphs for 15 odor-active compounds based on the different stimulus–response models (Fechner (Fechner, 1860), Stevens (Stevens, 1957), Hill (Hill, 1913; Chastrette et al., 1998) and found that with a proper calibration, up to nine different concentration levels can be discriminated by the panel (n = 8). The signal showed a good long-term stability, and its precision varied between 3.7 and 8%. They also found that the sensitivity to detect changes in concentrations of the compounds with GC-O is extremely dependent on the compound: in the best case, a concentration change of 20% can be detected, while in the worst, concentrations must differ more than one order of magnitude. According to Van Ruth 2004, intensity method resulted in higher discrimination between different concentration levels, but robustness of the detection frequency method was shown in better repeatability.

Van Ruth (2001) reported a review on different methods for GC-O. According to the author, the main drawbacks of the dilution techniques were the difficulty to use more than one assessor because of the lengthy process of the method and the invalidity of the two dilution factor assumptions. There is a nonlinear relationship

between the perceived intensity of a compound and its concentration and the slopes for different odour-active compounds are different. Koutidou et al. 2017 used both AEDA and detection frequency method to measure the importance of the odor-active compounds in tomato-onion purees and two methods resulted in different compounds to some extent – some of the compounds that resulted in high detection frequency value had low flavor dilution (FD) factor and also the opposite. This could be explained by differences in slopes of the concentration/odor intensity relationship, which means that different compounds present in twice as high concentrations than threshold value, may have totally different odor intensities. Therefore, according to Van Ruth et al. (2001a), detection frequency and posterior intensity methods gave better correlations between sensory intensities and compound concentrations compared to dilution techniques.

GC-O is nowadays used to solve different scientific and/or practical questions and problems. For example, it is used to select those compounds which are responsible for aroma defects in food, like different off-flavors and taints. The first ones are food-borne defects and the latter caused by contaminations. In both cases comparison with the reference product usually gives a limited number of sensory-relevant compounds to focus on (Blank et al. 2001).

Besides, according to Brattoli et al., 2013, GC-O studies on food products focus essentially on three key issues:
(1). The aroma profile of various foods and beverages and the dependence between the odor of food and the chemical composition of the volatile fraction on these products;
(2). The odor changes in food due to processing techniques (fermentation, cooking, the addition of preservatives and flavorings);
(3). The discrimination among a family of foodstuffs (cheese types, coffee)

For example, GC-O have been recently used to identify off-flavours in red wines (Pons et al., 2018) and beers (Noba et al., 2017). Red wines made from different quantities of *Plasmopara viticola* infected grapes were evaluated by using sensory analysis to describe the impact of the infection on the flavor profile of the end product. GC-O analysis was used to compare the profiles of infected and control samples and 6 potential odor-active compounds responsible for off-flavors were detected. 4 out of them were successfully quantified by using GC-MS. In case of the beer, onion like off-flavor was measured with using sensory analysis and GC-O to determine components responsible for the odor, which were finally quantified by using GC-MS. GC-O has also been used recently to determine aroma profiles and key aroma compounds in horseradish (Kroener et al., 2017), where AEDA was used to measure the importance of each compound, liquors (Niu et al., 2017), where also AEDA was used to determine 27 most important odor-active compounds that were quantified by using GC-flame photometric detection (FPD) and GC-MS, odor activity values (OAV) were calculated and recombination studies carried out; to evaluate the influence of different lactic acid strains on malt beverages by comparing AEDA results for 12 most important odor-active

compounds (Dongmo et al., 2017); to classify ciders based on their origin and maturation by using in parallel GC-O mean intensities and volatiles quantified with FID (Lobo et al. 2016).

2.1.1. GC- O Panel performance

The accuracy and reliability of GC-O analysis is very much dependent on the performance of GC-O assessors. Therefore, the selection and training of the panellist is of high priority. The literature available on training GC-O panellists, evaluating their performance and typical flaws and challenges of the GC-O analysis, is very limited. GC-O has a lot in common with sensory analysis, as human are used as detectors. A peculiarity of GC-O, compared to other sensory analyses, is to combine two discontinuous phenomena: the aperiodic and unpredictable elution of odor-active compounds from the chromatographic column and the breathing process (Hanaoka et al. 2000). Hanaoka et al. 2000, have investigated the effect of human breathing rhythm on the results of GC-O analysis and found that subjects with faster breathing cycle tend to detect odors more frequently and also to rate with higher intensities.

From one side, the job of a GC-O panellist compared to sensory analysis is easier as he doesn`t have to recognise different attributes from a matrix but rather detect and describe the intensity of pure aroma compounds. From the other side, GC-O panellist has only a limited time to detect, describe and quantify the odor without a chance to sniff it right again. Although there are many principle differences between GC-O and sensory analysis, the methods for analysing the performance of the sensory assessor and the panel could be applied to GC-O analysis.

There is a lot of literature available on the typical mistakes in sensory analysis. As these are mainly related to the scale usage and are therefore suitable to assess GC-O panel performance when the intensities of the compounds are involved.

According to Kernit et al., 2005, there are three different type of errors in individual level and four types in panel level;

<u>Individual errors:</u>

- Location error - the assessor uses a different location of the scale compared to the rest of the panel.
- Sensitivity error - the assessor is not able to discriminate between two or more products.
- Reproducibility error - the assessor is not able to consistently replicate a judgement for one or more products.

Panel errors:

- Magnitude error - the assessor uses a broader or narrower range of the scale than the rest of the panel.
- Crossover error - the assessor rates a product or set of products in the opposite direction from the rest of the panel.
- Non-discriminator error - the assessor rates all the products in a set as similar when the rest of the panel rated them as different.
- Non-perceiver error. The assessor does not perceive an attribute and scores all the products at '0' when the rest of the panel rated them as different.

Besides, fatigue and lack of motivation as well as concentration are very common sources for bias and can influence the GC-O results considerably. Though, careful selection and training of assessors will improve assessor performance, and therefore improve the accuracy and precision of the data collected. It is suggested that potential assessors should be screened for sensitivity, motivation, ability to concentrate, and ability to recall and recognise odor qualities (Delahunty et al., 2006).

Analysing the results of GC-O analysis can also be challenging as odor thresholds can vary significantly among individuals, and some people, with an otherwise normal sense of smell, are unable to detect families of similar smelling compounds, which is called partial anosmia (Brattoli et al., 2013). Therefore, evaluation of GC-O panellist should also include analysing the assessor's ability to sense specific compounds.

To the author`s best knowledge there is very limited literature available on evaluating the performance of GC-O panel.

2.2. Sensory analysis

Sensory evaluation is defined as a scientific discipline used to evoke, measure, analyse, and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste, and hearing (Stone et al.,1993). Though, instrumental methods are developing continuously in the field of simulating perception of food as human senses do, there is still no method available with capability to measure the human sensations as accurately as humans do. Therefore, sensory analysis is nowadays widely used even though it has many disadvantages caused by human fluctuations.

2.2.1. Quantitative descriptive analysis (QDA)

Descriptive analysis is the sensory method by which the attributes of a food or product are identified and quantified using human subjects who have been specifically trained for this purpose (Hootman, 1992). Descriptive analysis will usually have 8 to 12 panellists that would have been trained, with the use of reference standards, to understand and agree on the meaning of the attributes used. A quantitative scale is usually used for intensity which allows the data to be statistically analysed. There are several different descriptive analysis techniques used and, very often, combinations of different techniques are used. (Lawless et al., 2009).

QDA is a method developed by Stone et al., 1974. It starts with selecting the best assessors which show an ability to discriminate differences in sensory properties of foods they will be trained for. The next step - training, requires the use of reference or standard samples to stimulate the generation of terminology. Attention is given to development of consistent terminology, but panellists can have their own aspects on scoring in 15-cm line scale. QDA panellists evaluate samples one at a time in separate booths to avoid discussions. The results of QDA are statistically analysed and usually presented as spider-web diagrams. (Meilgaard et al., 2006)

2.2.2. Check all that apply (CATA)

CATA is a sensory method that is mainly used for consumer data. To carry out the analysis, a checklist is provided to the assessor and they are asked to tick the characteristics that apply to the sample. Sometimes the number of applied characteristics is limited (Lawless, 2013). Adams et al. 2007 proposed using CATA questions to allow consumers to indicate their sensory perception of the samples that were also being evaluated hedonically. CATA questions do not directly measure intensities. There is no scale to permit an assessor to characterize the level of difference. Nonetheless, evidence presented so far in the literature has shown good correlation between CATA frequencies and attribute intensities (Bruzzone et al. 2012, Reinbach et al. 2014). CATA questionnaires have been used increasingly in consumer research in the last few years (Laureati et al., 2017, Torres et al., 2017, Yoo et al., 2017)

A single CATA question including different terms could be presented to the panellists, or the terms could be separated into multiple CATA questions. The optimum number of terms used should be calculated carefully; fatigue effects and the duration of product evaluation should be considered. For example, if the panellists are asked to take one sip of a sample, their ability to describe the product will be dependent on their memory with all its limitations. Thirdly, the order of presenting the sensory terms should be decided. One approach could be to group

them to different questions based on the perceiving order or to group them under one CATA question according to the same strategy (Varela et al., 2014).

Typically, the first summary of CATA data is to determine the column sums of X, i.e., counting by product how many assessors checked the given attribute. Merging the different attributes yields the so-called contingency table. The values might be displayed as absolute counts or percentages. Generally, CATA tests are not carried out with replicates, but each panellist evaluates each product once. Afterwards, different data analysis approaches could be followed to evaluate the difference between the products across attributes and to present the data graphically (Meyners et al., 2013).

2.3. Chemometrics

The term chemometrics refers to applying advanced mathematical methods and algorithms to chemical data (Poole, 2012). It is applied to design the experiments, to gain maximum relevant chemical information by analysing chemical data and to obtain knowledge about chemical systems (Vandeginste et al., 1997). The changes and differences in aroma profiles of various products evoked by several factors as origin, processes etc. are hard to reveal without comprehensive and relevant information. Such information will almost invariably be multivariate in nature to comprehensively describe the underlying problems. Therefore, the need for advanced experimental planning as well as data analysis techniques is obvious (Marini, 2013). Though, statistical methods are helpful, it is important to emphasise that to get the most out of statistical design and analysis methods, one must use as much subject matter knowledge as possible. It is only when statistical and subject matter knowledge play well together that the best possible results can be obtained (Næs et al. 2010).

2.3.1. General statistical approaches in analysing GC-O data

Generally, in most of the studies involving GC-O analysis, the application of chemometrics to GC-O data, is quite limited. Commonly, the main aim is to find key odor-active compounds and to determine odor profiles of different food products and therefore results are presented as tables with detected odor-active compounds with an indication to their importance, either as intensity values (Lv et al., 2012), detection frequencies (Díaz-Mula et al., 2015), modified frequencies (Márquez et al., 2013; Egea et al., 2014) or dilution factors (Feng et al., 2015). In some of the cases, GC-O have been used as a technique to make a selection of sensorially relevant aroma compounds and the following statistical techniques like PCA is still carried out on the signal collected with GC-MS (Cheng et al. 2015).

Du et al. 2015, have followed a different strategy when investigating the aroma profiles of two different tomato cultivars harvested in 3 different stages. 50 odor-

active compounds were detected (scale from 1 to 15, 2 assessors) and the compounds with similar odor descriptors were grouped into five general odor categories based on their primary odor character: i) green/grassy/viney, ii) earthy/musty, iii) sweaty/stale/sulphurous, iv) fruity/floral, and v) sweet/candy. The odor profiles of tomato cultivars were presented and compared as spider-web diagrams based on the summed intensity values of compounds with similar descriptors grouped to the above-mentioned odor categories. The odor categories were ranked in the order of importance based on the summed intensity values of compounds in each category, although, descriptive sensory analysis was not carried out to compare the GC-O profiles with real perception of attributes. Akiyama et al. (2008) used Charm analysis to compare coffee from different origins and roasting methods and the assessors were told *a priori* which ten terms/categories to use for describing the odor quality. Afterwards, PCA was carried out on 36 variables having odor spectrum values (OSV) larger than 50 and also on total Charm values of each odor descriptor of 10 descriptor categories. Besides, PCA was also carried out on the GC-MS profile. Grouping based on the origin was similar for both GC-O/PCA and GC-MS/PCA biplot, but GC-O/PCA also enabled the differences caused by roasting conditions to be followed.

GC-O fingerprints have been used previously to classify Spanish ciders from two different regions and maturation stages (Lobo, et al., 2016). Classification was carried out by using partial least square – discriminant analysis (PLS-DA) and using in parallel two different data matrices – odor-active compounds quantified with GC-FID (nr. of variables=41) and mean relative intensities (I, %) for compounds determined with GC-O (nr. of variables 57). Volatile composition quantified with GC-FID provided satisfactory results ($R^2Y = 0.66$) only for modelling maturation of ciders, whereas the use of the olfactometric profiles as predictor variables allowed the ciders to be classified by both origin ($R^2Y = 0.77$) and maturation ($R^2Y = 0.90$).

Culleré et al. 2013, analysed GC-O modified frequency data (nr. of variables 44, MF > 30%) of different wood samples to evaluate their suitability for wooden barrels by a chi-square test (χ^2) (Pearson, 1900) to look for discriminating odorants. 15 odorants were chosen as the most discriminating, based on chi-square test and also including those volatiles which had a value of (MF_{max} - MF_{min}) higher than 60%. A principal component analysis (PCA) plot was constructed from the modified frequency scores of the most discriminant odorants, according to the χ^2-test (nr. of variables = 10). PCA was also applied by Olivares et al. 2013 on GC-O detection frequency data (nr. of variables= 42, all detected compounds) of fermented sausages together with some other chemical data like free fatty acids content.

2.3.2. Discrimination and characterization of honey samples by using GC-O analysis and chemometric tools

During the last decades, honey has been widely investigated, mostly in terms of authenticity control of unifloral or mono-varietal honeys. In honeys with mixed botanical origins, a honey type with strong sensory characteristics can be more dominant than mild honey types even at low proportions and change the overall sensory profile of the honey (Piana et al., 2004). Moreover, pollen content measured by traditional melissopalynological analysis dealing with microscopic investigation of bee honey does not reflect the actual botanical origin in full extent as the pollen in the nectar of different plants maybe over or underrepresented, because the bee`s honey stomach is prefiltering pollen from the nectar in different amounts. Also, the beekeepers tend to filter honey prior selling (Briant, Jr. et al., 2001). Therefore, sensory characterization of the honey may not necessarily match the botanical origin determined with pollen analyses. This has motivated researchers on finding new methods and techniques besides traditional pollen analysis to identify the biological origin and to classify honey samples based on their sensory properties. Plant-derived aroma compounds have been studied as indicators of the botanical origin by GC-MS (Castro-Vazquez et al., 2009; CastroVazquez et al., 2007; de la Fuente et al., 2007; Guyot et al., 1998; Guyot et al., 1999; Jerković et al., 2009; Piasenzotto, et al., 2003). However, the composition of flavour compounds of honey depends on several factors, like honey maturity, geographical origin, honey bee`s metabolism and technical processing of honey. Therefore, the identification of volatile marker compounds of unifloral honeys are generally difficult (Siegmund, 2017). Very limited data is available on searching marker compounds or characterization of different honey samples by using GC-O analysis. Pino, 2012 analysed the most important odor-active compounds in black mangrove honey by using AEDA method.

The classification of honey samples of different botanical origins has been carried out by using GC-MS and different multivariate statistical techniques. For example, Aliferis et al. 2010, used *m/z* fragments of SPME-GC-MS analysis for non-targeted analysis to classify honeys of different botanical origins by using orthogonal partial least square - discriminant analysis (OPLS-DA) and orthogonal partial least square – hierarchical cluster analysis (OPLS-HCA) methods. The level of misclassification was as low as 1.3%. Baroni et al. 2006 used HCA and stepwise discriminant analysis (SDA) to determine a group out of 35 VOCs measured by HS-SPME-GC-MS representing similitude and differences among studied 5 botanical origins. Thus, six out of 35 VOCs were selected, verifying their discriminating power by K-nearest-neighbor (KNN), which afforded 93% correct classification.

To the authors best knowledge GC-O has not been used before to classify honeys of different botanical origins. However, the method has shown promising results for classification of ciders based on the origin and maturation, where GC-FID

volatile fingerprints failed (Lobo et al., 2016). This could be due to the possibilities to quantify important odor-active compounds that are present in very low concentrations and/or overlapping with non-odor compounds. In addition, GC-O is an analytical method that measures only the compounds that have odor-activity and are present above odor threshold values and has therefore an advantage compared to the other analytical techniques in classifying the samples according to distinctive sensory characteristics.

2.3.3. Correlation of GC and sensory data by using PLSR

Investigating the relationship of the sensory perception of food with its volatile chemical components enhances the understanding of the flavour of any food. Although high correlation between volatile components and sensory attributes may not refer to a causal connection, it is indicating that the variables are changing in the same manner. For example, if the sample contains a high level of a measured volatile component it may be an indication of a high intensity of a sensory attribute with which it is correlated (Owusu et al., 2013). Sensory data and instrumental measurements are related in a variety of contexts and this serves a number of objectives. The most common academic use is to investigate the mechanisms by which physical properties of foods act to produce specific sensations during viewing, smelling or eating. This is also of interest to the food industry. Sometimes, the objective is to establish which sensory attributes can be accurately predicted by instruments, or a combination of instruments to improve online quality assurance (Macfie & Hedderley, 1993). Nowadays, various statistical methods are used to correlate sensory and instrumental data and to create prediction models with high statistical performance. Most of the times GC-MS is the analytical method for measuring volatiles compounds for correlating with sensory data. The summary of the recent practices in the field of correlating sensory and volatiles data are gathered to a table in appendix A. In last seven years, various articles were published on correlation of GC-MS and sensory data applied for wine (Xiao et al., 2014, Robinson et al., 2011, Green et al., 2011), cheese (Ochi et al., 2012) and other food products (Mimura et al., 2014, Viljanen et al., 2014). There are also studies where GC-O have been used as variable selection method to detect important odor-active compounds which are quantified with GC-MS and the latter are also used as the input for statistical analysis (Niu et al., 2011, Niu et al., 2017, Liu et al., 2015). Though, more often correlations with sensory data are studied by using GC-MS, there are some studies using GC-O as the input data for regression analysis (Michishita et al., 2010, Morita et al., 2015; Thomsen et al., 2012).

In statistical data analysis, the liability and usefulness of the results are dependent on the entire process, starting from the method for data collection and pre-processing to validation of the results. Macfie & Hedderley (1993) published a review on correlation of sensory and instrumental data focusing specifically on statistical methods used for correlation, not the entire process starting from data

collection. Recently Zielinski et al. (2014) reviewed and demonstrated the use of chemometrics in assessing different properties of fruit juices and summarised the overall features, advantages and disadvantages of different chemometric tools that could be applied to experimental data. This review was not specific to gas-chromatography or sensory analysis, but covered various aspects of chemometric analysis, including pre-processing and validation steps.

2.3.3.1. Variable selection

Variable selection is used to get better correlations between explanatory and independent variables and to improve the performance and prediction capability of the model. With many variables being irrelevant, noisy or unreliable, removal of these will typically improve the predictions and/or reduce the model complexity (Andersen et al., 2010).

Variable selection could be based on statistical techniques or, more subjectively, based on the prior knowledge of the variables. For example, Liu et al. (2015) used the flavour dilution factor according to AEDA analysis procedure to determine predominant odour-active compounds. Another possibility is to set a value of odour detection frequency (Bansleben et al., 2009) for which compounds below the threshold are excluded. The same approach has been used for the sum of posterior intensities (Thomsen et al., 2012) and the modified frequency method (Campo et al., 2005). The most often used statistical approach for variable selection is variable importance for the projection (VIP) value, which was first introduced by Wold et al. (1993). The VIP score for the variable j is defined as:

$$VIP_j = \sqrt{\frac{p}{\sum_{m=1}^{M} SS(b_m \times t_m)}} \times \sum_{m=1}^{M} w_{mj}^2 \times SS(b_m \times t_m) \qquad (2)$$

where p is the number of variables, M the number of retained latent variables, w_{mj} the PLS weight of the j-th variable for the m-th latent variable and $SS(b_m \times t_m)$ is the percentage of y explained by the m-th latent variable. The VIP value is namely a weighted sum of squares of the PLS weights (w), which considers the explained variance of each PLS dimension (Cassotti et al., 2017). Since the average of squared VIP scores equals 1, greater than one rule is generally used as a criterion for variable selection (Chong et al., 2005). For selecting the variables from sensory data, analysis of variance (ANOVA) has been used to get an overview of which variables have statistically significant differences among samples (Niu et al., 2011), but also to exclude the variables with no statistical difference among samples (Liu et al., 2015; Mimura et al., 2014). Also, it should be determined if the analysis involves only odor or also aroma/flavor attributes. Aprea et al. (2012) found that the models built with flavour attributes were less stable and gave poor results. The authors explained that flavour is an interaction between volatile

compounds, taste and texture; thus, they only presented the model with odour attributes.

2.3.3.2. Data pre-processing

To find structures in a data set or to reveal similarities of objects, the features need to be comparable (Bansleben et al., 2009). Therefore, data pre-processing is an essential part of chemometric data analysis. It can be separated into two main directions: removing data artefacts and transforming/rescaling the data by using a function. The most widely used method in data pre-processing is autoscaling (Bansleben et al., 2009; Mimura et al., 2014; Niu et al., 2011). This combines mean centering and standardisation (dividing with standard deviation); thus, it gives equal weight to each variable. It is important to autoscale the data, especially in the cases where data are in different units and/or large deviations in the matrixes are present. Otherwise, more importance could unintentionally be given to the variables that have higher values or bigger fluctuations between the samples in terms of absolute values.

2.3.3.3. Partial least square regression (PLSR)

PLSR is a method for relating two data matrices, X and Y, and uses latent variables to model the covariance of matrixes X and Y. PLSR can analyse data with noise, collinearity and missing variables in both X and Y matrices. It also does not require the number of samples to be higher than the number of variables. When increasing the number of relevant variables with the PLSR method, the precision of the model parameters improves (Wold et al., 2001). Due to the abovementioned benefits, PLS is nowadays the most widely used method to correlate sensory and instrumental data.

In terms of sensory and instrumental correlation, sensory attributes are the dependent variables (Y), that are predicted by the model. Volatile components are explanatory variables (X), which are used as an input data to define sensory properties of food. For each sensory attribute, a model could be built separately (PLS1) or the model could be built for all attributes together or for a group of specific attributes (PLS2). For example, Wold et al. 2001 suggested that PCA could be run on sensory attributes to determine highly correlated ones and then to run PLS on each correlated group of variables. For interpretation purposes, it is usually advantageous to use all *Y*-variables simultaneously, but for obtaining good predictions, the best choice is often to treat each variable separately (Næs et al. 2010).

The number of components included in PLSR analysis is usually determined by cross-validation (CV). CV is performed by dividing the data in a number of groups

and then developing a number of parallel models from reduced data with one of the groups deleted (Wold et al., 2001).

The goodness of fit of the models is evaluated by using coefficient of determination which is denoted as R^2 and measures the total variation explained by the model (Schroeder et al., 1986). To assess the prediction quality Q^2 is calculated which shows the goodness of prediction. Those are calculated as following:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2} \quad (5)$$

$$Q^2 = R^2_{CV} = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_{i/i})^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2} \quad (6)$$

Where y_i is the experimental value, $\bar{y}_i$ the average of experimental values, $\hat{y}_i$ the value calculated by the model and the $\hat{y}_{i/i}$ the value predicted by the model (Todeschini, 2017)

Another widely used estimate of the magnitude of the absolute error of the model is the root mean square error (RMSE), which could be similarly calculated for both calculated and predicted y values.

$$RMSE = \frac{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2}{n} \quad (7)$$

RMSE value is dependent on the weight and has the same units as variables. Therefore, it is not suitable to compare the quality of the models with different input data, but rather to compare the performance of different models applied on the same data.

3. AIMS OF THIS DISSERTATION

The main objective of this doctoral work was to investigate the possibilities to improve interpretation and evaluation of the GC-O data by using different traditional chemometric techniques. As the literature on methodological aspects on GC-O analysis as well as the published research by using GC-O methods together with chemometric techniques is limited, this thesis aims to look through the available approaches and bring some new insights to the possibilities of applying GC-O for different scientific objectives. To get reliable data, performance of the panel is crucial, therefore, the methodology to monitor GC-O panel performance was developed (Publication I). Secondly, the possibilities to use GC-O data together with AHC fingerprinting for differentiating honeys from different botanical origins were investigated, as well as characterizing the potential sensory properties of honey samples based on GC-O analysis (Publication II). As the main importance of the volatile compounds of food are related to the sensory perception they are causing, statistical aspects and methods for correlating volatiles data (GC) with sensory attributes were investigated through comprehensive research on recent practices (Publication III). Gathered knowledge was applied on correlating sensory CATA data with GC-O results by using PLSR analysis (Publication IV).

4. MATERIALS AND METHODS

4.1. Materials and chemicals

Commercially available kvass (Kvass original, A le Coq, Tartu, Estonia), used for panel training was purchased from local store in Estonia. 13 honey samples (Publication II) were collected from local beekeepers in Estonia and botanical origin determined by melissopalynological analysis. Samples 1 and 2 were unifloral raspberry honeys, 3–5 unifloral rape honeys, 6–8 honeys with high rape pollen content, 9–10 unifloral heather honeys, 11 honeys with high heather pollen content and 12–13 honeys with high alder buckthorn pollen content. Samples 12–13 could also be unifloral honeys, but there is no literature available determining the minimum content of pollen of alder buckthorn in unifloral honey. Samples 12 and 13 were visually rather different from other samples because of their dark colour and liquid consistency. Second set of honey samples for (Publication IV) were gathered form local beekeepers in Finland.

Sniffing strips used in panel training were bought from Orlandi Inc. (Farmingdale, NY, USA). All solvents, salts, reference compounds, and standards of chromatographic grade were purchased from Sigma-Aldrich (St. Louis, MO, USA), Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland). Vanilla essential oil was purchased from a local market. Ethanol, whenever used, was acquired from Rakvere Piiritusetehas (Rakvere, Estonia). Water was purified with Millipore (Millipore Corporation, Bedford, MA) whenever samples were prepared. A fragrance materials test mix (FMTM) was acquired from Restek (Bellefonte, PA)

4.2. Methods

4.2.1. HS-SPME-GC-O

For the analysis of Reference mixture A, 0.01 ml of the mix was injected into a 20 ml headspace (HS) vial that contained a 1-cm glass covered magnetic stir bar. Original FMTM (fragrance materials test mix) was diluted in ethanol (1 µl/ml concentration). 0.01 ml of the diluted mix was injected into a 20 ml HS vial containing a stir bar. 50% w/w dilution with water was made for all honey samples. For Estonian honey samples 1 ml of diluted honey together with 1 g of NaCl and for Finnish honeys 2 ml of diluted honey without NaCl were measured into a 20-mL SPME vial with a glass covered stirrer. Blossoms were placed into 20-mL SPME vial immediately after harvesting depending on the size of the blossoms, covering approximately 1 cm above the bottom of the vial. To apply the same headspace volume to all the samples and to avoid cutting the flowers, volume of the samples was used instead of the weights.

For sample preparation and injection, a CTC CombiPAL auto-sampler (Chromtech, Germany) with SPME option was used. The incubation time was 5 min at 60 °C for standard mix and honey samples and 35°C for blossoms, after

which a 2-cm SPME fiber (50/30-μm DVB/Car/PDMS Stableflex, supelco, Bellefonte, PA, USA) was injected into the vial for 20 min at 60 °C for standard mix and honey samples and 35°C for blossoms for extraction. Volatiles were desorbed in an Agilent 7890 gas chromatograph equipped with a flame ionization detector (FID) and a sniffing port ODP-3 (Gerstel, Germany). The column effluent was split 1:1 between the FID and the sniffing port using deactivated fused silica capillaries (1-m length, 0.15-mm i.d.) for training mix and Estonian honey samples, for Finnish honeys the column entered directly to sniffing port. The sniffing port was supplied with humidified air at 30 ml/min. The transfer line temperature was 300 °C. A capillary column DB-5MS (30 m 0.25 mm 1.0 μm; J&W Scientific, Folsom, CA, USA for panel training, Agilent Technologies, Santa Clara, CA for Estonian honey samples and flowers and Restek, Bellefonte, PA for Finnish honey samples) was used in the GC. Helium gas (purity 5.0, AGA, Estonia) was used as a carrier at a constant flow of 2 ml/min. Splitless mode was used in a split/splitless injector 250 °C. The initial oven temperature was 35 °C followed by a rate of 45 °C/min to 85 °C, then by 9 °C/min to 200 °C and then by 45 °C/min to 280 °C and held for 1 min (total run time 16.6 min).

In GC-O analysis for panel training and Finnish honeys posterior intensity scores were collected using scale 1 to 5. In case of Estonian honeys detection frequency method was used.

4.2.2. HS-SPME-GC-MS

For GC-MS analysis of the honey samples the sample preparation and extraction were carried out the same as for GC-O analysis. Volatiles were desorbed in GC–MS (Agilent 6890; Agilent Technologies, Santa Clara, CA) with a column of DB5-MS (30 m 0.25 mm 1.0 μm; J&W Scientific, Folsom, CA, USA for panel training, Agilent Technologies, Santa Clara, CA for Estonian honey samples and flowers and Restek, Bellefonte, PA for Finnish honey samples). The GC–MS was equipped with a time of-flight detector (Waters, Manchester, UK). For GC–MS data analysis the NIST05 library was used.

4.2.3. Sensory evaluation

For the sensory analysis of Finnish honeys, untrained panellists were given seven blind-coded, randomized honey samples, approximately 15 ml each, in lid-covered 100 ml glass vials to capture the headspace. The panel consisted of 62 panellists (35 females, 27 males) of age 15–71 years (mean 36.8 ± 13.2 y).

Sensory evaluation was carried out by using check all that apply (CATA) method. The main categories (12) of odor and flavor descriptors were berry-like, fruity, floral, herbaceous, woody, nutty, spicy, caramel, earthy, microbiological, chemical and animal-like, featuring 147 descriptors in total. Besides, the intensity of the odor, flavor, aftertaste and color together with sweetness, acidity and the

familiarity of the odor and flavor were evaluated on a 5-point scale from 1 (not at all) to 5 (very strong). Finally, questions related to honey consumption and preference were asked.

The data was collected using Compusense® five version 5.2 data collection software (Compusense Inc., Guelph, ON, Canada). Tests were conducted in controlled sensory laboratory conditions in accordance with ISO8589:2007 standard.

4.2.4. Monitoring of GC-O panel performance

GC-O panel (n=10) performance was evaluated in 7 months' period with training intensity two sessions in a month. The first 4 months (eight sessions in total) the panel analysed reference mixture A (Ref. mix A), followed by 3 months (six sessions in total) analysing fragrance materials test mix (FMTM). Ref. mix A composed of 9 pure standards (2,3-Butanedione, 3-methylbutanol, hexanal, dimethylsulfide, isoamyl acetate, methional, benzaldehyde, 1-octen-3-ol, β-damascenone). The exact concentrations of standards in Ref. mix A are brought in Publication I. Posterior intensity method was used, which means that the panellists were asked to detect, describe and quantify odor active compounds.

It must be noted that after mixing the nine solutions, the Ref. mix A consisted of approximately 20 odorous compounds due to impurities. Because some impurities had a similar odor description as the standard compounds such as dimethyl disulphide (rotten cabbage), 1-octen-3-one, octanol (mushroom), and cinnamic acid (kvass), they were also counted as a signal (13 compounds in total). FMTM contained 12 pure standards (benzoic acid, benzyl salicylate, 1,8-cineole, trans-cinnamaldehyde, cinnamyl acetate, cinnamyl alcohol, ethyl butyrate, geraniol, hydroxycitronellal, D-limonene, vanillin and thymol). Vocabulary training for the FMTM analysis was carried out using pure standards diluted in ethanol. After every training, the results were introduced to the panellists together with a possibility to sniff those compounds from sniffing strips to memorize the odor and the correct description. Results from regular training sessions were documented and the performance profile of each assessor was composed based on a statistical spreadsheet analysis and Panel analysis using XLStat, Addinsoft, New York, NY. No data treatment was applied prior to the analysis.

4.2.5. Agglomerative Hierarchical Clustering (AHC)

To classify the honey samples, GC-O detection frequency values and Agglomerative Hierarchical Clustering (AHC) was used. Hierarchical algorithm builds a hierarchy of clusters. Agglomerative hierarchical clustering (bottom to the

top) starts with clusters each containing one single dataset and continues merging the clusters (Gan et al. 2007). Clustering was based on dissimilarities between groups by using Ward`s method. Ward method was proposed by Ward Jr. (1963) and Ward Jr. and Hook (1963) when they were seeking to form the clusters in a manner that minimizes the loss of information associated with each merging. Usually, the information loss is quantified in terms of an error sum of squares (ESS) criterion, so Ward's method is often referred to as the "minimum variance" method (Gan et al. 2007). Ward's method uses the Euclidean distance between centroids of the clusters and attempts to minimizes the sum of the squared distance of points from their cluster centroids (Mooi et al. 2011).

$$d_{ij} = \sqrt{\sum_{k=1}^{K}(y_{ik} - y_{jk})^2} \qquad (8)$$

In equation 2, d_{ij} is the Euclidean distance between data points i and j. In two- and three-dimensional space, this corresponds to the usual distance that can be measured with a ruler (Næs et al. 2010).

According to Ward´s method those objects whose merger increases the overall within-cluster variance to the smallest possible degree, are combined.

Detected odor-active compounds in honey samples (n=13) with detection frequency values higher than 33% (nr. of variables=46) were included in the AHC analysis. No processing was applied to the data prior analysis. AHC analysis was carried out by using XLStat, Addinsoft, New York, NY.

4.2.6. Correspondence analysis (CA)

GC-O detection frequency data was grouped according to odor descriptors prior to CA by summing up the detection frequency values of similar descriptors compounds. In total 20 new variables were observed. Correlations between attributes were found using Pearson Correlation Coefficient (p=0.05). Correspondence analysis (CA) was used to analyse aroma profiles of Estonian honey samples. CA is based on the analysis of the contingency table through the row and column profiles. The usual purpose in using CA is to graphically represent these relative frequencies in terms of the distance between individual row and column profiles and the distance to the average row and column profile, respectively, in a low-dimensional space. Distance is measured using the chi-square metric. The chi-square distance between row *i* and row *i'* ($i \neq i'$) is given by:

$$d(i, i') = \sqrt{\sum_j \frac{(p_{ij} - p_{i'j})^2}{p_{+j}}} \qquad (9)$$

where p_{ij} and $p_{i'j}$ are relative frequencies for row i and i′ in column j and p_{+j} is the marginal relative frequency, or "mass" as it is called in CA, for column j (Sourial et al., 2010).

4.2.7. Partial least square regression analysis

GC-O data of Finnish honeys was processed using modified frequency formula:

$$MF(\%) = \sqrt{F(\%) * I(\%)} \qquad (10)$$

PLSR analysis was carried out to determine the correlations between sensory attributes and volatile composition of Finnish honeys. For the PLSR analysis, 12 main sensory descriptor categories were used. The frequencies used for PLSR analysis were calculated by summing up all the single descriptors in one category and descriptor called attribute in general. PLSR was applied to all the 12 attributes together and on groups of highly correlated sensory attributes determined previously by PCA. All the data was autoscaled prior to statistical analysis. For the analysis, only the predictors with VIP value larger than 1 were included. Analysis were carried out by using software R 3.4.0, package "plsdepot".

5. RESULTS

5.1. Monitoring of GC-O panel performance

After preliminary trainings of potential GC-O panellists, 10 of them were chosen to be part of professional panel following continuing training and monitoring of the performance (Publication I). To evaluate each panellist`s performance, the percentage of correctly detected compounds for each training sessions were calculated and, based on that, tendencies were drawn (Figure 2 and 3). At this point a panellist succeeded if s/he detected the odor and provided the correct description. The amount of correctly detected compounds, shows the performance of the panellist in each session, expressed as percentage of correctly detected compounds. From the graphs, it could be seen if the performance was improving with each new session as the proficiency of the panellist increased. Also, a negative trend could be observed, which may refer to fatigue or drop in motivation. For example, for Ref. mix A, 3 assessors out of ten had a negative trend in detecting the compounds, for FMTM the number of detected compounds usually stayed the same during sessions or dropped (Figure 4, Publication I).

As seen from Figure 2, illustrating the trend for assessor 6, during the first couple of months the motivation was high and, with each session with Ref. mix A, the performance improved. After three months, the performance dropped which may indicate a loss in motivation. When starting again with new compounds mixture FMTM at the starting point motivation was high, but the loss in motivations occurred even sooner, after first month.

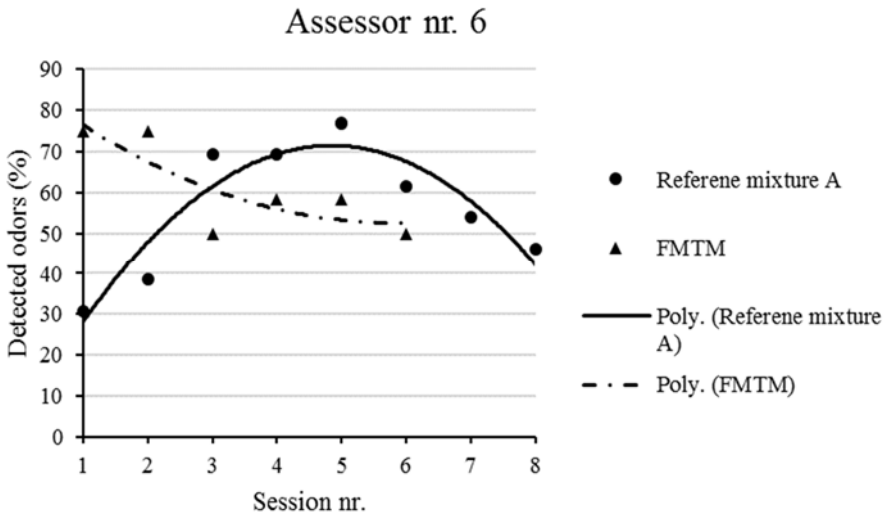

Figure 2. *Amount of correctly detected odors by assessor 6 for Reference mixture A and FMTM (Training started with Reference mixture A (4 months), followed by FMFTM (3 months)).*

On the other hand, assessor nr. 2 (Figure 3) showed no drop in motivation. She had very good and stable performance for Ref. mix. A. For FMTM, the percentage of detected compounds was lower but rather consistent excluding session 5.

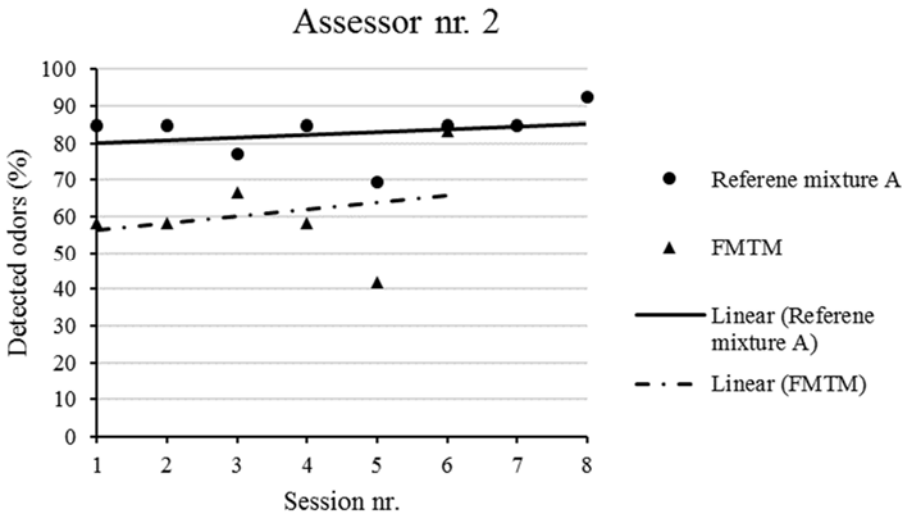

Figure 3. *Amount of correctly detected odors by assessor 2 for Reference mixture A and FMTM (Training started with Reference mixture A (4 months), followed by FMFTM (3 months)).*

From XLstat Panel analysis tool, information on detecting single specific compounds and the usage of scaling was observed. Figure 4 is an example for the panellists 1 and 2. There could be seen the average intensity score of all the sessions for each compound per each panellist and the total panel average score for each compound. The odor descriptors in the graphs are ordered according to their retention times. It could be observed which of the compounds were hard to detect for certain panellists to carry out more extensive training or confirm partial anosmia. From the Figure 4, also information on panel agreement and scale usage could be gathered. For example, panellist 1 rates higher whisky, potato and kvass compared to the panel average. For FMTM, panellist 1 tends to give higher intensity scores in case of almost all the compounds, so scale usage could need more training. It is also seen that assessor 1 fails to detect grass and mushroom compounds, which may indicate a partial anosmia or poor recognition of this compound. In FMTM more than one assessor did not sense citrus and flowery compounds and for example assessor 3 failed to recognize as much as 5 compounds in total, which besides above-mentioned causes, may also refer to the drop in motivation or insufficient physical or emotional conditions.

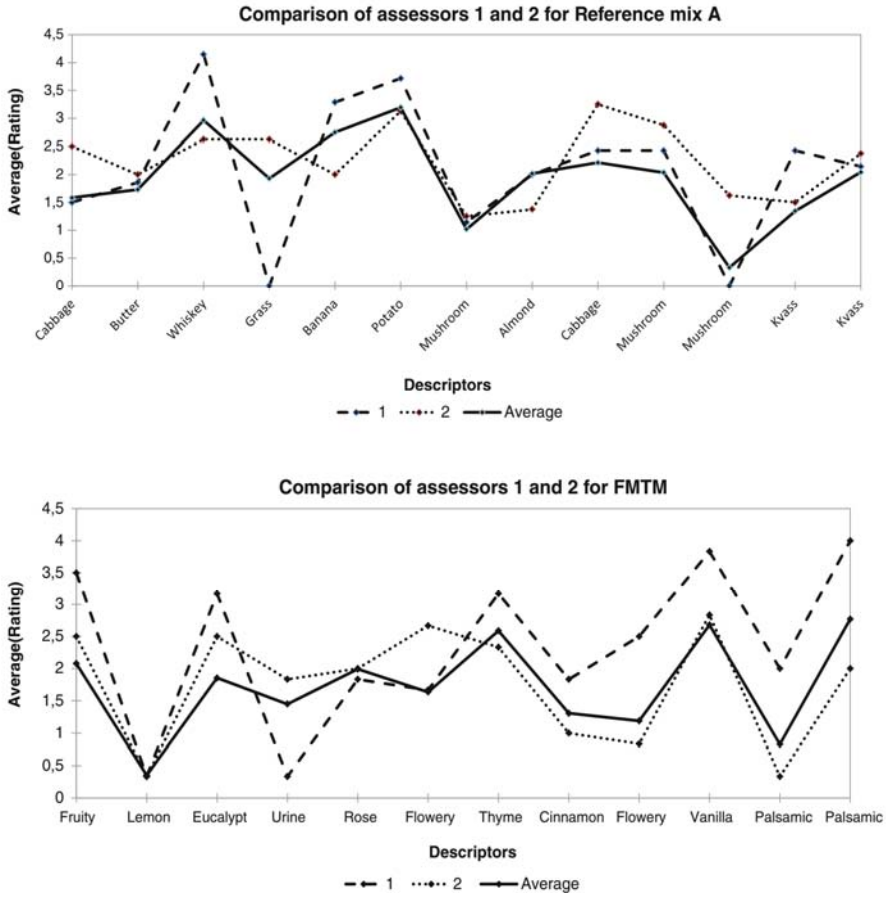

Figure 4. *Comparison of assessors 1 and 2 with panel (n=10) average in detecting and rating odors (scale 1 to 5).*

5.2. Classification and characterization of Estonian honey samples

Honey samples (n=13) were analysed with GC-O by using 3 assessors and detection frequency method. All the assessors sniffed the samples in duplicate resulting in 6 analyses in total. Detection frequency method estimates the importance of each aroma compound based on the number of sniffers detecting specific odor, based on which percentages were calculated. If the detection frequency value of the compound was larger than 33% it was counted as a signal, which means that the compound was detected 2 times out of 6 analyses. In total 46 odor-active compounds were detected with a detection frequency above 33 %. 18 of them were present in all the samples and 2 of them (isophorone and 2-methylbutyric acid) were found only in honeys of specific botanical origin (heather

honeys). The data matrix of 13 objects (honey samples) and 46 variables (aroma compounds) was subjected to AHC using XLstat software. As seen from the Figure 5, honey samples were clustered based on their botanical origin determined with pollen analysis.

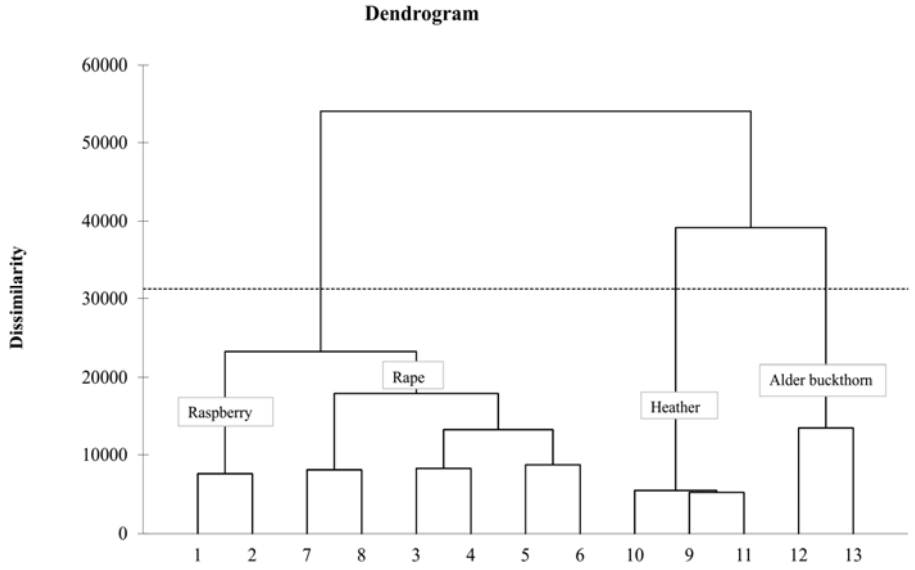

Figure 5. *AHC analysis of 13 Estonian honey samples based on GC-O detection frequency values (1-2 raspberry, 3-8 rape, 9-11 heather and 12-13 alder buckthorn honeys).*

Heather honey samples had the smallest internal dissimilarity measurement (see Fig. 5), which may indicate to the stable sensorial quality of heather honeys less dependent on geographical origin. Raspberry honeys have similarities with rape honeys, which could be explained by small amounts of raspberry pollen found in rape honeys. Heather honeys group together with alder buckthorn, again there were small amounts of alder buckthorn honey present in heather honeys as well Aliferis et al. (2010) used HCA on GC–MS data and obtained very good classification results of different honeys according to their botanical origin. Present study shows that GC-O detection frequency profiles also give sufficient fingerprints of honeys to correctly classify them by using HCA. Correspondence analysis (CA) was used to assess the flavour profiles of different honeys and based on the odor descriptors from GC-O (Figure 6). Although sensory analysis of the honeys was not carried out, by summing up the detection frequency values of similar odor descriptions into one category, it was aimed to get an overview of possible sensory characteristics that dominate in specific honeys. In total, 20 new variables were gained (like fruity, floral, herbal etc.). Similar approach has been followed by Du et al. 2015, who divided 50 odor-active compounds into 5 attributes what were used to compose profiles of different tomato cultivars.

CA compares the similar patterns in samples and therefore the closer the samples are in the biplot, the more similar their profiles. In general, correspondence analysis was suitable for assessing the flavour profiles of different honeys, showing similar grouping of honey samples as in case of AHC, though instead of the frequencies of single compounds, summed up categories were used. First two components explained the variance/inertia of the data well, having a value of 63,38 %.

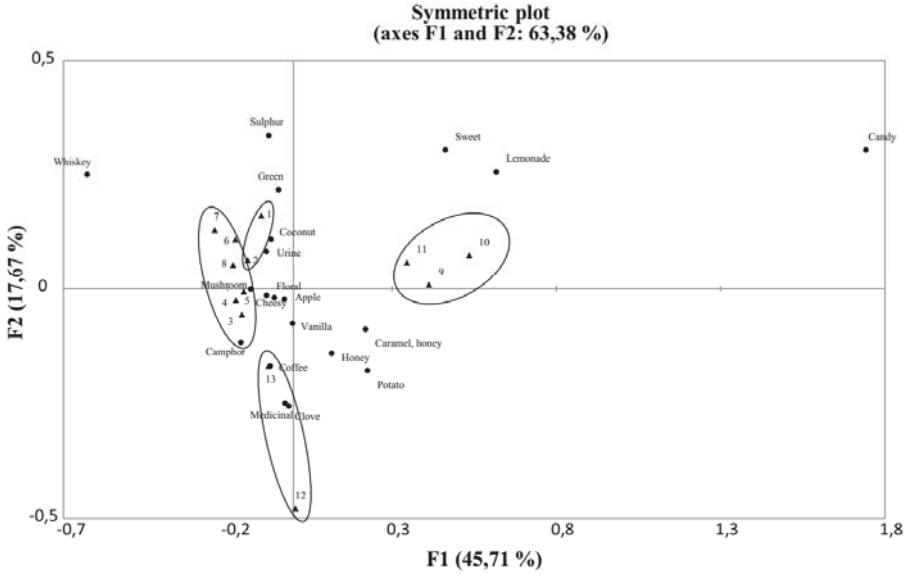

Figure 6. *Correspondence analysis of 13 Estonian honey samples (1-2 raspberry, 3-8 rape, 9-11 heather and 12-13 alder buckthorn honeys).*

Heather honeys had more odor-active compounds than the other investigated samples and could be described as having more sweet candy-like aromas. Raspberry honey can be characterised by a larger number of green notes and lack of honey notes. Rape honey has the poorest aroma profile without many characteristic notes as also mentioned by Plutowska et al. (2011). The only important feature in rape honeys as well as blossom is the sulphur attribute. Rape blossom seems to be the source for sulphur and all the samples contain rape pollen to some extent, which explains sulphur in the aroma profiles of most of the samples. Alder buckthorn honeys tend to have more floral and honey notes and less green and sweet/candy characteristics. Additionally, sulphur was not present (over threshold), unlike in the other honey samples.

5.3. Current practice in correlating data from gas chromatographic analysis with sensory properties of food

A comprehensive study was carried out on analysing the recent practices in the field of correlating volatiles data with sensory properties of food (Publication III). As the data, available on GC-O is very limited, the review covered also aroma profile analysis carried out with other detectors, mainly MS (Supplementary table 1). Descriptive analysis is the most frequently used technique to gather sensory data, as it provides the most accurate sensory profile, mostly due to the usage of trained assessors. In some cases, also CATA method has been used as it has an advantage when using untrained sensory panel or fast profiling is needed.

Autoscaling is the most commonly used pre-processing technique, VIP for variable selections and PLSR for regression analysis.

The study revealed that too often there is very limited information available on the chemometric aspects of analysing the results. That's also the case in data pre-processing techniques, where quite often the information on applied methods were not available. The information of the validation to evaluate the reliability of the data turned out to be inadequate in multiple cases.

5.4. Correlating sensory and GC-O data of Finnish honeys by using PLSR

Profiles of 7 Finnish honeys of different botanical origins were analysed by using untrained panellist (n=62) and CATA method. Sensory data from CATA analysis were grouped according to 12 main categories and only the odor descriptors from CATA data were included for correlation with GC-O. Sensory data was presented as frequency values for 12 attributes Besides odor descriptors (Table 1.) each category also contained the choice "odor in general", which frequency was also added to the total frequency of main category.

Table 1. Odor descriptors and 12 general categories used in CATA analysis.

Category in general	Odor/flavor descriptors in CATA
Berry-like	Strawberry, Raspberry, Blackberry, Currant/cassis, Blueberry, Lingonberry, Cloudberry, Cranberry
Fruity	Tropical fruit, Citrus, Lemon, Orange, Grapefruit, Lime, Banana, Apple, Pear, Cherry, Pineapple, Peach, Mango, Apricot, Melon, Guava, Dried fruit, Raisin, Prune, Fig, Date, Jam
Floral	Rose, Honeysuckle, Peony, Lavender, Lilac, Violet, Hyacinth, Dandelion
Herbaceous	Fresh herbs, Grass, Clover, Mint/peppermint, Menthol, Eucalyptus, Dried herbs, Tea, Malt, Tobacco, Hay/straw, Dried grass
Woody	Resinous, Beeswax, Pine, Spruce, Birch, Oak, Cedar, Burnt, Roasted, Ash, Coffee, Smoky
Nutty	Almond, Peanut, Walnut, Hazelnut, Pecan, Coconut, Chestnut
Spicy	Black pepper, Cinnamon, Ginger, Licorice/aniseed/ fennel, Clove, Nutmeg, Saffron
Caramel	Chocolate, Confectionary, Marshmallow, Vanilla, Maple syrup, Toffee, Treacle/molasses, Cotton candy, Burnt sugar, Brown sugar
Earthy	Wet earth, Mushroom
Microbiological	Lactic / lactic acid fermentation, Moldy, Cheesy, Yeasty, Baked bread
Chemical	Astringent/mouth-drying, Sharp, Pungent, Medicinal, Metallic, Alcoholic, Solvent, Sulfur, Cabbage, Cooling
Animal	Leather, Barnyard, Goat/caprylic, Sheep/wool, Dog, Sweaty, Cat urine, Locker room

In total 72 odor-active compounds was detected with GC-O by using posterior intensity method. Modified frequency values for each compound were calculated (Formula 1). All the detected compounds were included to the statistical analysis (Table 2).

Table 2. Compounds and their modified frequencies according to GC-O (405-buckwheat, 229-willowherb, 703-lingonberry, 197-cloudberry-bog, 689-sweet clover, 323 and 624-multifloral granular and smooth)

	Compound	Kovats RI[a]	Observed RI	Ident.	Fl. Description	405	229	703	197	689	323	624
1	dimethyl sulphide	505	516	St, MS, L	sulphur	49	29	39	35	24	13	0
2	1-propanol	536	541	L,	pungent	0	0	21	57	11	0	29
3	2,3-butanedione	593	599	St, L,	Butter	28	24	49	41	49	41	47
4	acetic acid	600	616	L	vinegar	42	45	47	38	39	22	18
5	3-methyl butanal	650	662	MS, L	Malty	82	37	39	41	0	9	61
6	methyl-2-methylpropanoate	685	674	L	Floral	28	0	11	13	0	0	0
7	methyl thiocyanate	685	686	L	roasty, onion	0	0	0	38	39	0	71
8	3-pentanol	759	760	L	Fruity	32	11	24	13	18	15	0
9	2-methyl-2-pentanol	768	765	L	Cheese	47	22	13	28	32	18	21
10	1-hexen-3-ol	789	778	L	Grass	0	0	21	13	0	0	15
11	(Z)-2-penten-1-ol	783	784	L	Plastic	42	47	21	44	53	52	24
12	2,3-butanediol	805	805	MS, L	Fruity	0	18	54	18	53	71	47
13	butyric acid	820	824	St, MS, L	Cheese	89	84	86	87	88	85	88
14	ethyl-2-methylbutanoate	846	838	L	Apple	58	52	52	49	54	46	39
15	ethyl-3-methylbutanoate	854	853	MS, L	Fruity	68	58	57	41	68	75	29
16	methyl-2-(methylthio)acetate	894	883	L	Roasty	73	68	70	63	68	65	73
17	ethyl pentanoate	900	902	MS, L	Fruity	33	0	32	0	0	18	0
18	Heptanal	903	909	L	Fat	0	34	0	57	0	47	29
19	Methional	909	920	St, L	Potato	39	11	26	33	29	13	26

	Compound	Kovats RI[a]	Observed RI	Ident.	Fl. Description	405	229	703	197	689	323	624
20	unknown 1		930		Cheese	52	21	58	69	73	25	75
21	methyl hexanoate	934[c]	937	L	Fruity	11	0	0	0	0	0	37
22	Heptanol	962	955	L	Green	42	15	0	28	11	0	13
23	1-octen-3-ol	982	983	St, L	mushroom	73	63	77	44	77	77	75
24	1-octen-3-one	976	986	L	Metal	77	80	84	69	86	82	77
25	ethyl hexanoate	1002	996	MS, L	Fruity	0	0	0	0	0	28	0
26	Methyldihydrothiophenone	998	998	L	Roasty	84	49	82	80	85	77	88
27	unknown 2		1011		Hay	0	0	0	0	0	0	55
28	α-phellandrene	1007	1013	MS, L	Herbal	65	75	58	38	53	35	55
29	p-cymene	1027	1032	MS, L	solvent	0	0	0	72	15	0	71
30	d-limonene	1030	1058	MS, L	Mint	13	21	34	25	37	0	35
31	Phenylacetaldehyde	1049	1065	St, MS, L	Honey	91	71	84	80	68	80	82
32	z-linalool oxide	1074	1071	MS, L	Floral	26	11	24	46	7	41	24
33	p-cresol	1074	1077	L	Urine	71	32	24	63	21	9	49
34	z-3-nonenal	1096	1098	MS, L	Green	0	0	0	0	24	0	18
35	3-hydroxy-4,5-dimethyl-2(5H)-furanone	1107	1102	L	caramel	89	86	89	89	89	87	89
36	Isophorone	1117	1112	L	Herbal	0	0	0	89	0	0	60
37	unknown 3		1123		Animal	86	65	62	0	73	75	69
38	2-phenylethylalcohol	1118	1125	St, MS, L	Flower	28	11	0	15	24	33	21
39	unknown 4		1138		malty, sour	53	32	0	35	75	15	71
40	lilac alcohol B	n.a	1144	MS, L	honey, floral	0	0	26	15	14	31	26

	Compound	Kovats RI[a]	Observed RI	Ident.	Fl. Description	405	229	703	197	689	323	624
41	(E,E)-2,6-nonadienal	1162	1152	St, MS, L	Green	13	34	21	25	7	13	21
42	lilac aldehyde A	1154	1158	MS, L	flowery	37	26	26	31	21	13	24
43	E-2-nonenal	1162	1162	MS, L	Green	37	86	69	69	68	63	82
44	2-phenylethylthiol	1176	1168	L	Rubber	86	77	77	49	71	59	77
45	ethyl benzoate	1185	1184	MS, L	Honey	0	29	63	71	32	35	68
46	unknown 5		1195		Roasty	39	34	51	28	47	69	60
47	E-linalool oxide	1212	1202	MS, L	Herbal	49	18	7	0	49	0	67
48	isobutyric acid	1215	1213	L	Dry	13	49	32	0	34	13	80
49	benzothiazole	1240	1230	L	flowery	43	68	58	72	39	35	47
50	ethyl phenylacetate	1252	1239	St, L	Honey	89	77	69	85	73	63	75
51	phenylacetic acid	1262	1250	L	Honey	21	39	37	0	73	25	47
52	Citral	1254	1258	L	Citrus	11	24	34	72	37	38	26
53	d-carvone	1265[c]	1267	L	Thyme	77	71	79	80	77	77	52
54	p-anisealdehyde	1275[c]	1283	L	aniseed	56	26	61	67	11	59	62
55	γ-butyrolactone	1299	1301	L	Honey	49	47	18	9	58	55	0
56	unknown 6		1305		Metal	0	0	13	0	11	69	21
57	3-phenylpropanoic acid	1321	1323	L	Herbal	86	77	77	41	81	82	84
58	ethyl 3-phenylpropanoate	1351	1354	L	Floral	0	0	56	9	0	15	0
59	unknown 7		1360		Cellar	65	71	49	59	66	0	26
60	Eugenol	1364	1368	MS, L	Clove	52	62	49	57	48	72	52
61	hexyl hexanoate	1379	1385	MS, L	Apple	89	80	77	88	24	36	17

	Compound	Kovats RI[a]	Observed RI	Ident.	Fl. Description	405	229	703	197	689	323	624
62	E-β-damascenone	1386	1400	St, L	Apple	80	87	88	76	47	75	73
63	Vanillin	1410	1420	St, L	Vanilla	80	85	88	76	47	75	73
64	unknown 8		1430		Dill	11	11	45	59	0	0	13
65	geranyl acetone	1448	1448	L	Herbal	11	52	34	25	34	25	0
66	β-caryophyllene	1467	1466	MS, L	Woody	52	26	49	58	68	59	49
67	ethyl cinnamate	1460[c]	1484	L	cinnamon	73	73	66	69	73	69	58
68	ethyl laurate	1493	1494	L	Dill	32	32	21	72	66	51	45
69	methyl dodecanoate	1509	1513	MS, L	Dill	29	11	21	0	24	0	18
70	z oak lactone	1538	1537	L	aniseed	0	0	21	38	11	13	7
71	hexyl octanoate	1566	1559	L	peppermint	68	66	62	57	32	44	42
72	(E)-whiskey lactone	1629	1633	L	chamomile	26	11	13	15	0	0	0
73	γ-dodecalactone	1685	1687	L	Herbal	7	21	0	21	0	0	0
	Total modified frequency					**2946**	**2636**	**2893**	**3047**	**2784**	**2590**	**3070**

[a]www.flavornet.org. [b]basis for identification: St, standard compound; MS, GC–MS; L, literature (RI).
[C]www.pherobase.com

Sensory data (Y variables) and modified frequency values of 73 volatiles (X variables) were subjected to PLSR analysis to find correlations between sensory odor attributes and volatile compounds. PLSR was carried out for all sensory attributes together as well as correlated groups determined with PCA to improve the explained variance and regression model's quality.
To determine correlations between Y variables PCA was carried out (Figure 7).

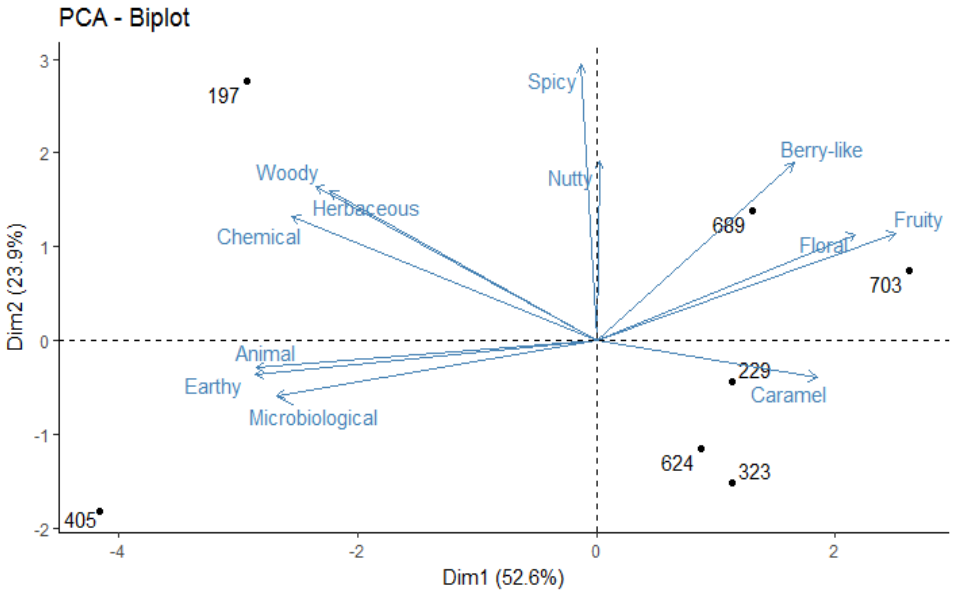

Figure 7. *PCA Biplot of 12 sensory categories*

It was evident that PLSR worked better on groups of correlated attributes instead of modelling all sensory attributes at once. Model quality indicators are brought out in Table 3.
For PLSR analysis of each group, only volatiles with high importance (VIP > 1) to the model were included. This downsized the number of variables to each model to 27-40 volatiles. For all the grouped attributes, the variance explained by the model was from satisfactory to good, with 5 components for 3 groups (A, C, D) the quality was excellent.

Table 3. Variance explained (R^2Ycum) and predicted (Q^2cum) by using cross-validation (CV) by the PLSR models

Group	Group description	R^2Ycum (2 comp)	Q^2cum (2 comp)	Q^2cum (5 comp)
A	Nutty/Spicy	0.88	0.55	0.99
B	Berrylike/Fruity/Floral	0.82	0.7	0.77
C	Herbaceous/Woody/Chemical	0.96	0.66	0.93
D	Earthy/MB/Animal	0.97	0.8	0.92
	All attributes together	**0.68**	**0.27**	**0.41**

In Figure 8, biplots for each group may be observed. Caramel attribute has been left out from the analysis as it could not be modelled with satisfactory quality even when modelled alone. For the PLSR biplots each volatile is marked with a number marked in Table 2.

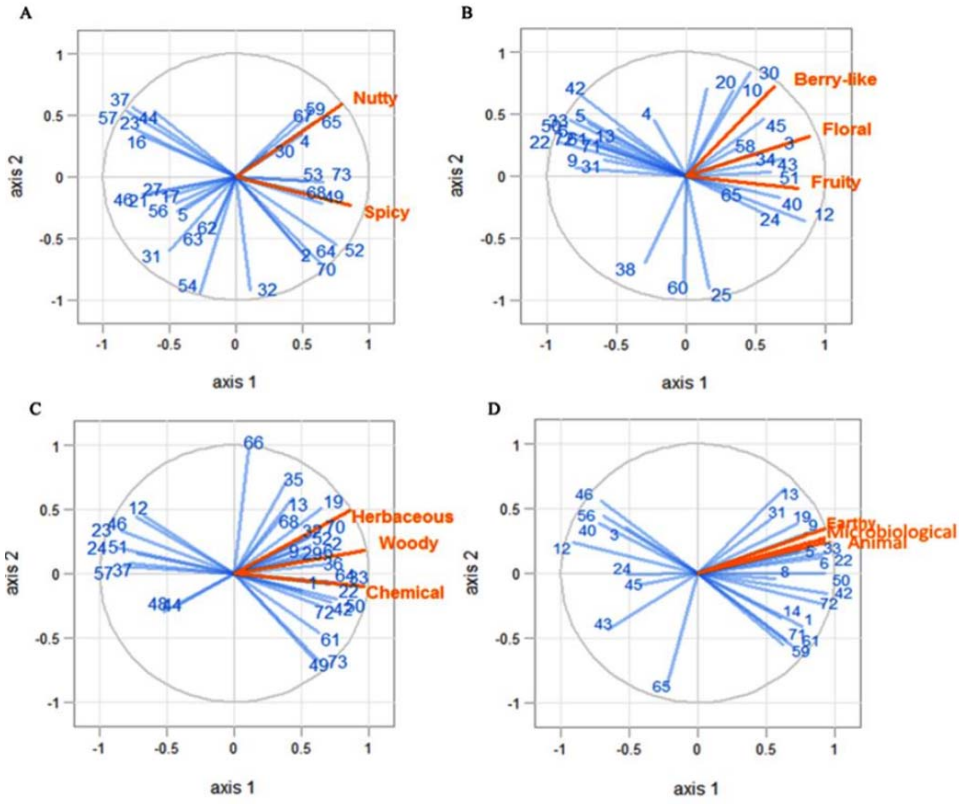

Figure 8. *PLSR correlation biplots of 4 groups of eleven sensory categories and odor-active compounds (explained X-variance 52-65%, Y variance 82-97%).*

The strongest positive correlation was observed between attributes in group D, which also had a strong positive correlation with multiple volatile compounds with similar odor descriptions as butyric acid (cheese- and faecal-like) (V13), *p*-cresol (cow- and barn-like) (V33). Also, 3-methylbutanal (malty) (V5) heptanol (herbal) (V22), methional (potato) (V19) and 2-methyl-2-pentanol (cheesy) (V9) have a strong correlation with animal-like, microbiological and earthy aroma, which were characteristic to the buckwheat honey (405). As also seen from the PCA plot group D have a strong negative correlation with group B, especially fruity attribute. Therefore, it is expectable from PLSR plot to indicate that the absence of volatiles responsible for specific animalic notes result in rise in fruitiness. On the other hand, fruity and floral notes have positive correlations with variables 2,3-butanediol (fruity) (V12), lilac alcohol B (floral) (V40), phenylacetic acid (honey-like) (V51), E-2-nonenal (green) (V43) and 2,3-butanedione (butter) (V3).

1-propanol (pungent)(V2), *p*-cymene (solvent)(V29), isophorone (herbal)(V36) and citral (citrus)(V52) had strong positive correlation with woody, but also with other group C attributes. Woody notes were mostly characteristic to cloudberry-bog honey (197). Besides, methional (potato)(V19) and Z-oak lactone (aniseed)(V70) revealed a high positive correlation with herbal notes according to PLSR.

In group A, nutty attribute has the strongest correlations with geranyl acetone (herbal) (V65) and ethyl cinnamate (cinnamon) (V67). Spicy notes are best explained by citral (citrus)(V52), unknown 8 (dill)(V64) and z oak lactone (aniseed)(V70).

6. DISCUSSIONS

Though, there isn`t much information available on evaluating GC-O panel performance, methods and practices of evaluating descriptive sensory panels could be adopted also for GC-O panels. That in terms of evaluating the repeatability and the ability to differentiate between samples as well as scale usage to some extent. Though, in GC-O analysis assessor must evaluate considerably larger number of different variables compared to traditional sensory analysis and therefore the capability to detect the variables/odor areas should be addressed in different manner. Constant monitoring of how is the number of detected compounds and the scale usage changing over time, gives a good indication, weather the assessor is consistent and motivated to perform the tasks.

Panellists´ ability to detect specific compounds was mostly increasing in the start of the trainings with Ref. mix A, which is logical as the proficiency was rising. In case of FMTM, almost opposite trend was seen. As by the time assessors started sniffing FMTM, their proficiency had been risen and this is well seen from the fact that in the first session with FMTM, the number of detected compounds was very often higher than in the first session with Ref. mix A. Unfortunately, in many cases a negative trend could be observed, which indicates to fatigue and loss of motivation. Loss of motivation and loss in alertness may have occurred as the perceived compounds were already common, the perceived intensities were rather low (the panel average intensity scores were below 3 in case of almost all the sniffed compounds) and the number of the compounds in the training mixes were quite low (13 and 12). Van Ruth et al. (2001b) brought out the above-mentioned factors as having an influence on the decrease in alertness and consequently also on number of detected compounds. The graphs of the amounts of detected compounds illustrate well the challenges in GC-O analysis, though, assessor is physiologically capable of detecting specific compounds due to the physical or mental fluctuations the repeatability of the results is a significant issue.

The classification of honeys based on botanical origin is very complex matter and often not so straightforward due to the different representation of pollen in honeys. Honeys with very distinct sensory characteristics referring to certain botanical origin may have very low correspondent pollen content. Therefore, a lot of research has been carried out to find specific volatile compounds - marker compounds, which characterize different unifloral honeys to help determination of honey according to botanical origin. It has not been very successful as often the markers proposed by one authors are often withdrawn by the others when the same compounds have been also found from honeys of the other botanical origins. Therefore, more promising approach could be non-targeted analysis, also by analysing the GC-O fingerprints of different samples. According to this study, GC-O was revealed as potential method for non-targeted profile analysis. As GC-O measures the volatiles that have odor activity and are present in concentrations above sensory threshold, it has a potential in classifying the samples with distinctive sensory differences and botanical origins.

Using Correspondence analysis (CA) to characterize samples measured with detection frequency method and grouping the similar odor descriptors provided the same clustering of honey samples as according to pollen analysis and gave a possibility to find potential sensory notes common to specific groups. CA helped to find similar patterns between the samples. This means that the absolute number of summed frequency for every descriptor` class was not as relevant in terms of analysis when the relative proportions. The samples were grouped together if they had the same order of importance for the descriptors. As there is limited literature available on sensory properties of different Estonian honeys, sensory analysis should have been carried out to assess the results gained by CA in more detail.

When investigating the practice in correlating GC and sensory data it was evident that GC-O results is quite rarely used as input variables for correlation with sensory data. It is mainly used to determine odor-active compounds, but afterwards the identified compounds are quantified with other detectors. As one of the aims for correlating sensory and instrumental data is to investigate the possibilities to replace sensory analysis with instrumental ones, to get rid of the human fluctuations, it is obvious that GC-O is not fulfilling that aim. Therefore, correlating GC-O and sensory data will mostly serve a purpose of getting deeper insight on the mechanisms behind sensory cognition so it could be directed. Also, it was revealed that often there is not paid enough attention on the statistical tools used and the descriptions of used methods were in some cases inadequate. That was mostly noticed for applying any pre-processing techniques and also the results were presented without reliability indicators, like coefficient of determination. The missing information could be related to the fact that rather often the statistical analysis is carried out with software that have been precoded and therefore the final user runs the analysis without thinking of the applied procedures. The published review gives a very good overview for the people who are interested in the field of correlating sensory and GC or any other instrumental data by explaining which are the aspects and steps that should be paid attention and which methods have been used by the others for similar tasks.

For correlating the sensory and instrumental data, dividing the sensory attributes to correlated groups has a positive effect on model quality and helps in interpreting the results; Correlations between sensory attributes and specific volatiles could be observed more clearly. As seen from the result the CATA data of untrained panellists sufficiently enabled to correlate the data with GC-O results. Although, the panellists were untrained, they were familiar with honey samples and characteristics in general and therefore can recognise the sensory attributes in a reasonable manner.

Though, often sensory perception is a complex system and sensory notes could not be caused by the volatiles with same descriptors, in this study a lot of sensory characteristics had strong correlations with volatiles which logically explained the perceived notes. Based on the results it could be pointed out which are the key odor-active components for honeys from different botanical origins. Still, as the number of samples was limited, a more comprehensive study should be carried out to validate the results.

7. CONCLUSIONS

7.1. Conclusions from Publication I

To assess the performance of each GC-O panellist calculating the proportion of correctly detected compounds is a good indicator. The trend line based on the change of the percentage of correctly detected compounds shows the panellist`s performance over time. The scale usage and repeatability error of the panellists could be observed similarly to traditional sensory analysis. Quite often negative trend in detecting the compounds could be observed, which most possibly is caused by the fatigue and the loss in motivation. GC-O analysis are challenging as even if the assessor is physiologically capable of detecting specific compounds due to the physical or mental fluctuations the repeatability of the results is a significant issue.

7.2. Conclusions from Publication II

Using AHC to classify samples by using non-targeted analysis with GC-O detection frequency fingerprints is a promising approach. As an example, 13 honey samples from 4 different botanical origins were clustered similarly according to the pollen analysis by using GC-O detection frequency method.

Using correspondence analysis on variables gained by summing up detection frequency values of volatile compounds with similar odor descriptors enabled to group the samples based on botanical origin and gives a good indication on the possible sensory aroma profiles of different sample groups.

7.3. Conclusions from publication III

The literature review shows that although statistical methods are widely used to correlate sensory and gas chromatographic data, information on the treatments applied to the original data set and also the validation results are often inadequate or missing. There is limited information on different variable selection techniques used and very little research conducted on comparing the results gained with different techniques. VIP and ANOVA appeared as the most used methods. Moreover, PLSR is shown as the most common method for correlating and calculating the models of sensory and gas chromatographic data. In many publications, even when the models or correlation parameters are described, the indicators of the reliability of the results like the values of Q2 and RMSE have not been mentioned.

7.4. Conclusions from publication IV

PLSR performed on groups of correlated variables is a useful technique to investigate correlations between sensory and instrumental relations providing key odor-active compounds characteristic to specific honeys. CATA data on untrained panellist could be used for modelling correlations with instrumental data.

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ABSTRACT

Aroma is a very important characteristic of food, as it is having the main influence on how people perceive its quality. Aroma molecules have often rather low detection threshold which makes the detection and quantification of specific compounds in various matrixes a challenging task. Classical instrumental detectors may fail to assess the aroma profile of food in the manner that is similar enough to sensory perception of human assessor. Therefore, gas chromatography-olfactometry (GC-O) is a useful technique combining instrumental separation technique and human assessor as a detector. Although, GC-O enables to receive aroma profile of the samples with good sensitivity, it is coupled with different challenges. Humans, as assessors bring subjectivity and flaws caused by the variation in sensitivity between the panellists and inconstancy within the different runs of the same panellist.

Statistical methods are widely used in different disciplines to help extract valuable information and discover patterns in data matrices invisible for human sight. As the literature on methodological aspects on GC-O analysis as well as the published research by using GC-O methods together with chemometric techniques is limited, this thesis aims to bring some new insights to the possibilities of applicability of GC-O for different scientific objectives.

The accuracy and reliability of GC-O analysis is very much dependent on the performance of GC-O assessors. Therefore, the selection and training of the panellist is of high priority. In current study possibilities to monitor the GC-O panel performance are presented, to observe the improvement in performance and loss in motivation which will result in decrease of alertness. Scaling differences in intensity measurements can be analysed similarly to descriptive sensory analysis. Agglomerative hierarchical clustering (AHC) was applied in this study to classify honey samples from different botanical origin by using non-targeted analysis with GC-O detection frequency method. It revealed to be a promising approach as samples clustered similarly to botanical origin determined by pollen analysis. Using correspondence analysis (CA) on GC-O data, where detection frequency values for similar descriptors were summed up and new variables gained, gave a good indication on the odor profiles of different honey samples. When reviewing relevant studies, it was observed that partial least square regression analysis is widely used to correlate sensory and instrumental data. Though, information on the treatments applied to the original data set and the validation results are often inadequate or missing. Next, by applying partial least square regression (PLSR) on sensory check-all-that-apply (CATA) and GC-O data of Finnish honeys, it was revealed that, by applying models on groups of highly correlated variables, the quality of the regression models were increased remarkably. Correlation between volatile compounds and sensory descriptors of Finnish honey samples could be observed.

The studies have been carried out based on practical need in developing and applying chemometric approaches to GC-O tasks to provide the output of maximum information and high reliability. Different GC-O methods as well as chemometric techniques will be continuously applied to meet the challenges of food sector together with increasing scientific knowledge.

KOKKUVÕTE

Toidu kvaliteedi hindamisel on selle aroom inimestele üheks olulisemaks näitajaks. Aroomiühendid esinevad toidus aga tihtipeale väga madalates kontsentratsioonides mistõttu on nende tuvastamine ja kvantifitseerimine erinevates maatriksites keerukas ülesanne. Klassikalised instrumentaalsed detektorid ei suuda määrata toidu aroomiprofiili viisil, mis annaks piisava täpsusega edasi toidu tarbimisel tekkivat sensoorset aistingut. Seetõttu gaas-kromatograaf-olfaktomeetria (GC-O) on kasulik meetod, mis on kombinatsioon instrumentaalsest lahutusmeetodist ja inimese haistmismeelest kui detektorist. Kuigi, GC-O on hea tundlikkusega meetod toidu lõhnaprofiili määramiseks, kaasneb sellega ka mitmeid väljakutseid. Inimesed, kui detektorid, toovad kaasa subjektiivsuse ja vead, mis on põhjustatud indiviidide tundlikkuse erinevusest erinevate lõhnavate ühendite suhtes ning samuti ühe assessori tundlikkuse kõikumisest päevade lõikes.

Statistilised meetodid on laialdaselt kasutusel erinevates valdkondades, et aidata eristada olulist informatsiooni ja leida mustreid, mis palja silmaga on märkamatud. Kuna GC-O analüüsi metodoloogilisi aspekte, sh. kemomeetriliste meetodite rakendamist, käsitleva kirjanduse hulk on piiratud, oli käesoleva töö eesmärk pakkuda uusi võimalusi GC-O rakendamiseks erinevate ülesannete lahendamisel.

GC-O täpsus ja usaldusväärsus on suuresti sõltuv GC-O assessorite soorituse kvaliteedist. Seetõttu, assessorite valik ja treening on kõrge tähtsusega. Antud töö raames käsitletakse võimalusi GC-O assessorite soorituse monitoorimiseks, mis võimaldaks jälgida nii soorituse paranemist kui ka motivatsiooni kadu, mis omakorda vähendab assessorite keskendumist. Erinevusi intensiivsuste hindamisel skaala kasutuses on võimalik analüüsida sarnaselt klassikalisele sensoorsele analüüsile.

Erinevat botaanilist päritolu meede klassifitseerimiseks rakendati uurimistöös mitte-sihitud analüüsi kasutades sisendina ühendite detekteerimissagedust GC-O analüüsil ning rakendades aglomeratiivset hierarhilist klasterdamist (AHC). Kuna meeproovid klasterdusid sarnaselt õietolmu analüüsiga määratud botaanilisele päritolule võib antud lähenemist pidada üheks potentsiaalseks alternatiiviks mee botaanilise päritolu tuvastamisel. Meede aroomi iseloomustamiseks liideti sarnase lõhnaga aroomiühendite detekteerimissagedused, saades uued muutujad, mida kasutati sisendina korrespondentsanalüüsis (CA).

Uurimustöö raames viidi läbi kirjandusanalüüs selgitamaks viimase 7 aasta praktikaid sensoorse analüüsi ja gaas-kromatograafia tulemuste korreleerimisel, mille käigus selgus, et osaline vähimruutude regressioonanalüüs (PLSR) on kõige laialdasemalt kasutusel olev meetod antud vallas. Siiski, informatsioon algandmetele rakendatud eeltöötluse ning tulemuste valideerimise osas on tihtipeale märkimata või puudulikult esitatud. Järgnevalt rakendati PLSR meetodit Soome meeproovide sensoorse analüüsi (check-all-that apply) ja GC-O tulemuste korreleerimisel. Andmete analüüs näitas, et jagades sõltuvad muutujad (sensoorse

analüüsi atribuudid) eelnevalt gruppidesse vastavalt omavahelisele korrelatsioonile paraneb mudelite kvaliteet märkimisväärselt ning korrelatsioon sensoorsete atribuutide ja lenduvate ühendite vahel on täpsemalt jälgitav.

Antud uurimistöö ajendiks oli praktiline vajadus rakendada erinevaid kemomeetrilisi lähenemisi GC-O analüüsi tulemuste tõlgendamisel, et saada kätte maksimaalselt kõrge usaldusväärsusega informatsiooni. Erinevad lähenemised GC-O analüüsi kasutamisel koos statistiliste meetoditega leiavad pidevat rakendust toiduainetööstuse väljakutsete lahendamisel ja teadlaste kompetentsi tõstmisel.

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APPENDICES

Appendix A

Table S1. Examples of multivariate analysis on relations of sensory and gas-chromatographic data carried out since 2009.

	Sample	No. of samples	Extraction method	Instr. technique	Type of instr. data	Data pre-processing	Statistical method for correlation	Results	Model quality	Reference
1	Oregano	3	Stir-bar-sorptive extraction (SBSE)	GC/O/FID	NIF value / FID peak area	Autoscaling	PLSR, FA	For predicting the right class of specimen with PLSR for specimen 2, errors are 11% and 4%.	No data	Bansleben et al. 2009
2	Cherry wine	9	HS-SPME	GC/MS-TOF	Relative conc.	no	PCA, Correlation analysis	First 2 Principal Components explained 69,99% variance of volatiles and 97,61 % variance of sensory attributes. Reasonable conclusions could be drawn based on received correlation coefficients.	No data	Xiao et al. 2014
3	Australian Cabernet Sauvignon	30	HS-SPME	GCxGC/ MS	Relative conc.	Normalizing (each product both x and y variables) against maximum value	PLSR (PLS1), two-way HCA	4 PLS components explained 97-99% of y-variance. X-variance not well explained (from 8-77%). 303 volatiles clustered in 30 groups based on correlation coefficients.	No data	Robinson et al. 2011
4	Sake	40	SBSE/SE	GC/MS	Relative conc.	Autoscaling	OPLSR (PLS 1)	Explained variance of models were higher than 90%.	r_{cv} = 0,905-0,963	Mimura et al. 2014

	Sample	No. of samples	Extraction method	Instr. technique	Type of instr. data	Data pre-processing	Statistical method for correlation	Results	Model quality	Reference
5	Arabica Coffee	58	HS-SPME	GC/MS/ FID	Chromatograms	Alignments (COW method), Smoothing (Savitzky–Golay algorithm, autoscaling, mean-centered	PLSR	When using the number of latent variables determined from RMSECV values for all models, variance explained was in general 95 % in case of Y and 52 % for X-matrix.	RMSECV 0,18-0,39. r_{cv}= 0,88-0,91	Ribeiro et al. 2009
6	Pomelo juice	2	HS-SPME/SE	GC/MS /FID	Relative conc.	Standardization	PLSR	The first 2 PLSR componets explained 21-86 % of y-variance.	No data	Cheong et al. 2012
7	Sauvignon blanc wine	18	HS-SPME	GC/MS	Conc.	no information	PLSR	The first 2 PLSR componets explained 67 % of x-variance and 65 % of y-variance. Perceptual separation between different origins.	No data	Green et al. 2011
8	Fruit smoothie	12	HS-SPME	GC/MS	Relative conc.	no information	PLSR	Poor correlation, coefficients r < ± 0,3	No data	Keenan et al. 2012
9	Cherry wine	5	DCM extraction	GC/O/MS	Relative conc.	Autoscaling	Anova PLSR	The first 2 PLSR componets explained 63 % of x-variance and 59 % of y-variance. Mostly Pearson correlation coefficients > ± 0,5	No data	Niu et al. 2011
10	Semi-hard cheese	7	Purge and Trap	8W-GC/O	Sum of GC-O intensities	Mean-centering	PLSR	The first 2 PLSR componets explained 68 % of x-variance and 89 % of y-variance.	No data	Thomsen et al. 2012

	Sample	No. of samples	Extraction method	Instr. technique	Type of instr. data	Data pre-processing	Statistical method for correlation	Results	Model quality	Reference
11	*Makgeolly* (rice wine)	12	HS-SPME	GC/MS	Relative conc.	no information	PCA	8 volatiles out of 45 correlated with sensory attributes well. Pearson correlation coefficients > ± 0,7.	No data	Jung et al. 2014
12	*Godello* (white wine)	5	SPE	GC/MS	Relative conc.	no information	PLSR (PLS1 and PLS2)	The first 2 PLSR componets explained 98 % of x-variance and 51 % of y-variance. PLS1: y-variance was explained 44-79%.	RMSEP lower than 10 for all models	González-Álvarez et al. 2011
13	Bioprocessed lingonberry	6	HS-SPME	GC/MS	Relative conc.	Standardization, normalization	PLSR	The first 2 PLSR componets explained 88 % of x-variance and 90 % of y-variance.	No data	Viljanen et al. 2014
14	Hard and semi-hard cheese	13	SE	GC/MS, GC/FID	Relative conc.	Pareto scaling, auto-scaling, centering	PLSR (PLS 1)	Models for 2 chosen sensory attributes explained both more than 95% of y-variance.	RMSEP 0,136-1,248 and r_{cv} = 0,919-0,985	Ochi et al. 2012
15	Solaris white wines	12	DHS	GC/MS, GC/FID	Semi-quantified (relative peak areas)	Autoscaling	PLSR	The first 2 PLSR componets explained 44 % of x-variance and 78 % of y-variance.	No data	Liu et al. 2015
16	Red wine	57	SE/SPE	GC/FID	Conc.	no information	PLSR (PLS 1)	Satisfactory models (explained variance > 45%) gained half of the sensory attributes	RMSEP = 0,81-2,71 and r_{cv} = 0,2-0,81	Aznar et al. 2014

	Sample	No. of samples	Extraction method	Instr. technique	Type of instr. data	Data pre-processing	Statistical method for correlation	Results	Model quality	Reference
17	Walnuts	4	HS-SPME	GC/MS	Relative conc.	no information	PLSR	The first 2 PLSR components explained 45 % of x-variance and 51 % of y-variance.	No data	Lee et al. 2011
18	*Vitis finivera* red cultivars	5	SE/SPE	GC/FID, GC-ion trap -MS	Relative conc.	Standardization	PLSR (PLS 1)	In case of 4 important sensory parameters correlated to instrumental data, the explained variance of models were higher than 99,6 %.	No data	Vilanova et al. 2012
19	Pomegranate juice and wine	6	HS-SPME	GC/MS	Semi-quantification (Relative peak areas)	no information	PLSR	The first 2 PLSR components explained 86 % of x-variance and 80 % of y-variance.	No data	Andreu-Sevilla et al. 2013
20	Apple	18	HS-SPME	GC/MS	Relative conc.	Log-transformation, mean-centering, normalization	PLSR (PLS 1), HCA	Apples clustered in very similar groups based on sensory results and volatiles. Y-variance explained was 0,52-0,95%	R_{cv} = 0,52-0,95.	Aprea et al. 2012
21	Peach, nectarine	4	Dynamic HS	GC/FID	Conc.	Autoscaling	PLSR	Correlated in the same models besides volatiles also different physiochemical properties.	No data	Cano-Salazar et al. 2013
22	Chocolate	6	Dynamic HS	GC/MS GC/O/FID	Semi-quantification	Autoscaling	PLSR (PLS1 and PLS2)	PLS2 components 1 and 2 explained 86% of total variance. PLS1 best results got attribute fruitiness (80% explained variance)	RMSECV = 1,62 and r_{cv} =0,55	Owusu et al. 2013
23	Spanish Albarino wines	35	SE	GC/FID	Relative conc.	no information	Pearson correlation coefficient, PLSR	Only 5 Pearson correlation coefficients between sensory attributes and volatiles were larger than 0,5.	RMSEP = 9-14	Vilanova et al. 2010

	Sample	No. of samples	Extraction method	Instr. technique	Type of instr. data	Data pre-processing	Statistical method for correlation	Results	Model quality	Reference
26	Chocolate	9	SAFE	GC/O/MS	Conc.	no information	PLSR	The first 2 PLSR componets explained 97,8 % of x-variance and 88,3 % of y-variance.	No data	Liu et al. 2015
27	Beer	32	HS-SPME	GC/MS	Peak areas	Autoscaling	PLSR (PLS 1)	R^2 for two analysed sensory parameters were between 0,8851-0,9678	RMSECV = 0,25-0,33	da Silva et al. 2012
28	Espresso		Dynamic HS	GC/O	Charm values	no information	ANN, PLSR	Analysing 10 odor and 4 sensory descriptors with PLS R^2 was 0,55 and ANN R^2 was 0,79	With PLSR r_{cv}= 0,35 and with ANN $r_{cv=}$ 0,77	Michishita et al. 2010

PUBLICATION I

Vene, K., **Seisonen, S**., Koppel, K., Leitner, E., Paalme, T.

A Method for GC-Olfactometry Panel Training.

Chemosensory Perception, 6 - 4, 179-189, 2013.

Chem. Percept. (2013) 6:179–189
DOI 10.1007/s12078-013-9156-x

A Method for GC–Olfactometry Panel Training

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Received: 7 June 2013 / Accepted: 4 September 2013 / Published online: 29 September 2013
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Abstract Odor-active compounds are commonly analyzed using gas chromatography–olfactometry (GC–O). However, there are only limited guidelines available for panelist training with this technique. In the current study, 29 volunteers were trained to detect, describe, and rate the intensity of odors. In addition, three GC–O methods, i.e., aroma extraction dilution method, detection frequency, and posterior intensity (PI), were used to evaluate the newly trained panelists' ability to analyze key compounds of kvass (fermented nonalcoholic drink) aroma. A five-step approach is proposed for training as follows: (1) introduction of the method; (2) vocabulary training using standard compounds and learning the use of the scale; (3) training with the reference mixture A; (4) training with the real product of interest—the beverage kvass; and (5) monitoring and further training of the panel. Following these steps, all panelists learned how to perform GC–O analysis. Some variances among subjects were observed; however, the background of the trainees was found to be insignificant. Assessors for the "professional" GC–O panel were chosen for further training and included people with a sensory and food science background, but also ordinary consumers. The PI method, where subjects rate odor intensity after a peak eluted, was found to provide a sufficient amount of data for key compound analysis. The method enabled easy data handling, provided valuable feedback for panel monitoring, and aided in the selection process to decide which assessors would be suitable for further training and placement on a professional panel.

Keywords GC–olfactometry · Kvass odor · Panel training · SPME

Abbreviatons

AEDA	Aroma extract dilution analysis. Dilutions are used to reveal the most intense flavor compounds in the extract. This results in *D*-values showing the highest dilution at which a substance can still be detected by olfaction.
CHARM	Combined hedonic response measurement. Differs from AEDA in that the duration of an odor is taken into consideration in calculations of odor unit values. Also, dilutions are presented in randomized order to avoid bias introduced by knowledge of the samples.
DF	Detection frequency method. Estimates the odor intensity based on recording-detected odors from a number of sniffers. To compare detection frequencies, both NIF and SNIF are used.
FD	Dilution factor
FMTM	Fragrance materials test mix
GC–O	Gas chromatography–olfactometry
HS	Headspace
NIF	Nasal impact frequency. This parameter indicates the peak height of a summed aromagram expressing the percentage of detection frequency of the odor. Nasal impact frequency of 100 % means that the odor was detected by all assessors.
OT	Odor detection threshold
OID	Olfactory intensity device. A technique that affords tracking odor intensity vs. retention time.

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PI	posterior intensity method. The PI method involves recording the odor intensity on a scale after a peak has eluted from the column.
SMM	Single magnetic mixer
SNIF	Surface of nasal impact frequency. This parameter indicates the peak area of a summed aromagram expressing the percentage of detection frequency of the odor and the time duration of detection.
SNIFF	Program from Gerstel that summarizes olfactometry results of individual assessors into a group aromagram.
TI	Time-intensity method. The intensity of odor is rated with time-intensity device, providing an odor peak similar to a chromatogram.

Introduction

Gas chromatography–olfactometry (GC–O) refers to the use of human olfaction as a sensitive and selective detector for odor-active compounds separated using GC. The accuracy of GC–O is very much dependent on the performance of olfactory assessors (sniffers). Each chromatically resolved compound that emerges from GC that has a concentration higher than the odor detection threshold (OT) of a human assessor has the potential to have its odor detected, described, and intensity quantified by a human assessor. However, without prior training and practice, this is difficult.

Debonneville et al. (2002) analyzed a flavor model analysis with three different panelists and found large deviations in both the sensitivity and the ability to recognize different compounds. It is clear that assessors have different potentials to detect each compound, but a panel must be representative and reproducible. Both of these are heavily compromised if there is a lack of consistency in the way panelists have been trained.

At present, GC–O methods that quantify the potency or intensity of an odor can be classified into the following categories: dilution analysis [aroma extract dilution analysis (AEDA) and combined hedonic response measurement (CHARM), detection frequency (DF), posterior intensity (PI) and time intensity (TI) methods (van Ruth, 2001)]. Pollien et al. (1997) reported that DF, where only odor detection is conducted, does not require training. On the other hand, Van Ruth and O'Connor (2001) show that training is still beneficial, because it increases the sensitivity of the method by reducing the signal-to-noise level of the group of assessors. Le Guen et al. (2000) have also reported that detection frequency may be applied without panel training, but still selected a panel that had been trained in odor recognition and had experince in GC–O in this study.

Both training guidelines and standards have been published for sensory detection methods. For example, Chambers et al. (1981) compared a trained and a semitrained panel and proved that the use of a small highly trained panel is justified in descriptive sensory analysis. More recently, Del Castillo et al. (2008) describe methods of training, validation, and maintenance of a sensory panel with an objective of being able to discriminate between dry bean texture properties. Another example presents a case study of a panel trained for the sensory evaluation of carrots (Kreutzmann et al., 2007). This case study shows that for some attributes the learning process can be longer and training has a significant effect.

A limited number of guidelines have been proposed for GC–O methods. Delahunty et al. (2006) reviewed the use of GC–O methods and discuss the importance of panel training on the quality of the results. Hulin-Bertaud et al. (2001) trained eight panelists and evaluated their ability to describe blue cheese extracts using a time-intensity method. Another study by Van Ruth and O'Connor (2001) trained panelists over a 4-month period and observed an improvement in precision. Panelist training for the olfaction analysis of aroma extracts was described by Bianchi et al. (2009). While GC was not used in this study, the training process they describe may still be applicable to a GC–O panel as well.

Due to the inherent differences between GC–O and sensory methods, each requires different training procedures. Sensory techniques usually make use of trained panelists who taste the food and try to assess the intensity of flavor attributes (salty, sour, vanilla, etc.) It is possible to assess one attribute several times before scoring, if necessary. In GC–O analysis, odors are presented to the assessors for a few seconds at undefined intervals over a relatively long period (up to 20 min), requiring the panelist to react quickly. During sensory evaluation, the perceived flavor of the sample involves simultaneous tasting of all flavor chemicals, and therefore, accurate assessment of the level of a particular flavor chemical may not be possible and often is not the objective. Odor analysis using a GC/MS instrument in combination with GC–O may be used to separate the components of a sample composed of hundreds of chemicals while simultaneously determining the odor potential and quantity of each chemical.

The objective of this study is to develop a method to train and monitor GC–O assessors. An additional aim is to compare which GC–O method (AEDA, DF, and PI) is most robust when applying the method with an inexperienced panel. The assessment is based on the assessors' abilities to perform the task, reliability of results, time consumption, and complexity of data treatment. The panel is trained to analyze kvass, and thus we test the efficacy of the training method by comparing the ability of the panel to detect and describe the key odor-active compounds in kvass before and after training. To investigate the effect of expertise, panelists from a variety of backgrounds were selected.

Kvass is a refreshing nonalcoholic drink (less than 1 % ethanol) that is well known in Central and Eastern Europe and

made by simultaneous acid and alcoholic fermentation of rye bread, rye/barley malt, or rye flour with additional sugar. Fermentation is carried out using lactic acid bacteria and yeast.

Materials and Methods

Materials

2,3-Butanedione, 3-methylbutanol, hexanal, dimethylsulfide, isoamylacetate, methional, benzaldehyde, 1-octen-3-ol, beta-damascenone, benzoic acid, benzyl salicylate, 1,8-cineole, *trans*-cinnamaldehyde, cinnamyl acetate, cinnamyl alcohol, ethyl butyrate, geraniol, hydroxycitronellal, D-limonene, thymol were acquired from Sigma-Aldrich (St. Louis, MO, USA; Table 1). Vanilla essential oil was purchased from a local market. A fragrance materials test mix (FMTM) was acquired from Restek (Bellefonte, PA). Odor-active compounds used as standards were diluted with ethanol from Rakvere Piiritustehas, Estonia. Kvass (A. Le Coq AS, Tartu, Estonia) was purchased from a local store. Sniffing strips were bought from Orlandi (Farmingdale, NY, USA). MilliQ water (Millipore SAS, Molsheim, France) was used for making kvass solutions.

Selection of the Panelists

Twenty-nine volunteers (5 male) were trained to become GC–O assessors. Some had previous extensive sensory training ($n=10$, mean age 27.5 years); some had a food science background ($n=10$, mean age 25.5 years), while others were typical consumers ($n=9$, mean age 28 years). The panelists were all healthy nonsmokers with each trained in one GC–O method (AEDA, PI, and DF). The distribution of panelists with different backgrounds was equally but randomly distributed between the GC–O methods.

Training of the GC–O Assessors

Training was carried out in five steps: (1) introduction of GC–O methods to the panelists during 2 days of theory lectures; (2) vocabulary training using standard compounds and learning the use of the assessment scale; (3) training with reference mixture A by detecting and measuring the intensity of the components in the mixture with the GC–O; (4) sniffing the product of interest; (5) monitoring and further training of the panel twice per month using reference mixture A and commercially available fragrance material test mix (FMTM).

GC–O analysis of Kvass Using Untrained Assessors

To investigate the effect of training on GC–O analysis, we compared the sniffing results of kvass before and after the training. Only limited instructions were given to the assessors. Assessors were asked to detect an odor and describe it with his choice of words. Pretest samples were prepared as DF and PI samples (see below).

Introducing the Method

The GC–O method was presented to the panelists over two days in a lecture format. An overview was given on human senses, odor activity of chemical compounds, odor sensation, and threshold. Panelists were introduced to sample preparation procedures, GC, including comprehensive and two-dimensional GC, mass spectrometry, and GC–O. In addition, they were introduced to the following GC–O methods: (1) AEDA CHARM, (2) DF, (3) TI, and (4) PI methods.

Table 1 Standard Compounds Used for GC–olfactometry Panel Training

Compound	CAS number	Odor perception	Odor threshold (mg/m^3)[a] Air	Odor threshold (mg/kg)[a] Water	Stock solutions in EtOH (g/L) Concentration	Reference mix A in EtOH (g/L)
2,3-Butanedione	431-03-8	Butter	0.00001–10.2	0.0003–2.3	1.2	0.07
3-Methylbutanol	123-51-3	Whiskey, malty, burnt, pungent, balsamic, alcohol, fruity, ripe, bitter	0.019–6.3	0.7–70	2.8	0.16
Hexanal	66-25-1	Grass, tallow, fat, fruity, fishy, herbal	0.025–0.098	0.0091–0.75	2.8	0.16
β-damascenone	23726-93-4	Apple, rose, honey	0.000002–0.00004	0.0000075–0.01	1.21	0.7
Methional	3268-49-3	Cooked potato	0.000063–0.06	0.0002–0.0018	1.02	0.6
Dimethylsulfide	324-92-0	Cabbage, sulfur, gasoline	0.003–3.5	0.00016–0.09	1.07	0.06
1-Octen-3-ol	3391-86-4	Mushroom	0.012–0.028	0.000005–0.025	1.1	0.06
Isoamylacetate	123-92-2	Banana	0.004–8	0.002–2.5	0.98	0.06
Benzaldehyde	100-52-7	Almond, burnt sugar	0.014–4.3	0.072–111	1.22	0.07

[a] Van Gemert (2003)

Panelists were familiarized with the test protocol which included avoiding alcoholic drinks, very spicy meals, and coffee before sniffing. During the session external disturbances were minimized and no communication between assessors was permitted during the session. Assessors were instructed to be calm and take their time traveling to and preparing for each session. The panel leader announced the time of each session early enough to allow for advanced planning and used regular times/dates for the sessions. Assessors should not sniff over 6 samples/day and not over 15 min at a time to avoid fatigue. In the case of longer GC runs, it is suggested to split the run between two assessors, but then at least two parallels with different switching times must be carried out.

The panelists were advised to breathe normally, preferably quicker (Hanaoaka et al. 2000). During sniffing, the panelists were asked to focus on stronger odors to avoid extensive noise detection quite common to inexperienced assessors. Also, they were not shown the chromatogram while sniffing to avoid imagining the appearance of a smell as the GC peak appears.

Training Using Standard Compounds

The second step introduced the odor vocabulary and assessment scale to the panelists. Nine commercially available aroma compounds found in kvass were used to train the assessors (Table 1). During training, it is recommended to use compounds that are present in the product of interest. The concentrations, odor thresholds (Van Gemert, 2003), and perceptions (according to www.flavornet.com and www.pherobase.com) of the standard compounds are also given in Table 1. Approximately 1 cm of the sniffing strip was dipped into the stock solution and sealed into screw-cap test tubes after evaporation of ethanol for sniffing (approximately 10 s at room temperature). Sniffing strips of one compound at a time were used to learn the vocabulary (each assessor's native language was used, mostly Estonian, but also Russian). Assessors memorized all nine odors and their descriptions and were subsequently tested with the same compounds. The panelists were required to describe the odor after sniffing the indexed paper strip only once. Solutions with standard compounds were stored for a maximum of 5 months because aldehydes are not stable in ethanol and form acetals.

For approximate and tentative intensity measurements, different sniffing strips were prepared. Benzaldehyde solution (Table 1) was diluted 100 times for intensity 1 (0.0122 g/L); isoamylacetate solution was diluted ten times for intensity 2 (0.098 g/L); hexanal was diluted two times for intensity 3 (1.4 g/L); β-damascenone solution (1.21 g/L) was used for intensity 4 and 3-methylbutanol (100 g/L) was used for intensity 5. Sniffing strips were dipped into the corresponding reference solution, the excess ethanol subsequently evaporated and the strips were placed into screw-cap tubes. In a group discussion session, assessors rated the perceived intensities of the compound on a five-point intensity scale using only integers (1 = very weak, not identifiable; 2 = weak, but identifiable; 3 = moderate, easily recognizable, but not strong; 4 = strong; 5 = extremely strong) as suggested by Ferreira et al. (2003) and Berdague et al. (2007).

GC–O Training with the Reference Mixture A

The third step consisted of the analysis of reference mixture A. Reference mixture A was composed of 0.1 ml of each solution (Table 1), diluted by ethanol 1:1 with a total concentration of 0.06 to 0.16 mg/mL (Table 1, last column). It must be noted that after mixing the nine solutions, the reference mixture A consisted of approximately 20 odorous compounds due to impurities. Because some impurities had a similar odor description as the standard compounds such as dimethyl disulfide (rotten cabbage), 1-octen-3-one, octanol (mushroom), and cinnamic acid (kvass), they were also counted as a signal (13 compounds in total); other odorous impurities were ignored in the data analysis.

Reference mixture A, consisting of previously memorized standard compounds, was sniffed three times by each assessor: two times to detect and recognize the compounds, and a third time to determine the intensity of a compound. Correctly detected peaks are counted when the assessor detects them at least two times. Correctly recognized peaks are counted, when the assessor recognized it at least one time giving exactly the odor quality he had previously memorized.

Assessors were given individual feedback on their performance by comparing individual aromagram data to the average group aromagram including information on intensity scaling, the number of peaks detected, and the number and suitability of descriptors given. It was possible for the panelists to use the sniffing strips in between the sniffing sessions to refresh their memory.

GC–O Training with the Real Product

The fourth step was to train the panelists with the real product, kvass, which is more complex and includes odors unfamiliar to the panelists. Panelists were divided randomly into three groups that each applied a different olfactometric method: DF ($n = 10$, mean age 29 years), AEDA ($n = 10$, mean age 26.5 years), and PI ($n = 9$, mean age 26 years). The result from one group was treated as one signal and the signal-to-noise level was chosen as two (meaning that at least three assessors had to detect the odor), as suggested by Van Ruth and O'Connor (2001).

Group 1, which used a DF method, analyzed kvass (0.5 ml in 20 ml vials, described in "Sample preparation and instrumental analysis" section) in duplicate and recorded both the beginning

and the end of the odor exiting the GC; they did not assess the odor quality. Kvass was not diluted and was adjusted in strength by volume such that about 30 odor compounds were perceivable to the sniffers (Reineccius 2010). The number of sniffers (n = 10) detecting an odorant in kvass was either tabulated and plotted in nasal impact frequency (NIF) values using a spreadsheet (% of assessors detecting the odor) or combined for a surface of nasal impact frequency (SNIF) chromatogram representing olfactory intensity device (OID) values [% frequency × duration (s)] using the Gerstel SNIFF program as described by Plutowska and Wardencki (2008). If an assessor failed to register a gap between two closely eluting compounds, they were nevertheless separated into two signals in the spreadsheet.

Group 2 used an AEDA method to analyze kvass in four dilutions: (1) 3 ml kvass and 1 ml of water in 20 ml vial [dilution factor (FD) = 1.33], (2) 1 ml of kvass and 3 ml of water in 20 ml vial (FD = 4), (3) 0.1 ml kvass and 3.9 ml water in 20 ml vial (FD = 40), and (4) 0.01 ml kvass and 3.99 ml water in 20 ml vial (FD = 400). This group detected odor-active compounds and also recorded the odor description. Results are given in log(FD) value, which is calculated by taking the logarithm of FD of the last dilution at which the signal was above noise level. Alternatively, using the Gerstel SNIFF program, results are given in OID values that take into account percent of frequency, duration and FD.

Group 3 used PI to analyze kvass (0.5 ml in 20 ml vial) in duplicate and recorded the maximum odor intensity as well as description once the compound had eluted. A remote control was used to express the intensity of a compound by pressing buttons from 1 to 5 (1 = very weak, not identifiable; 5 = extremely strong). Both the SNIFF program and a spreadsheet program were used to analyze the results. Using the spreadsheet, the parallel results of each assessor were summed in a table (retention time; odor impression and intensity). All the compounds were sorted in order of retention time. If a compound was detected in both parallels, it was highlighted and not repeated in the list. Afterwards, the results of all assessors were listed together and grouped according to retention time and odor impression. Intensity values were averaged.

Monitoring and Further Training of the Panel

The goal was to train and finally choose at least eight assessors for the "professional" GC–O panel to be further trained and monitored. We tentatively call the panel professional because the panel may be called such after a minimum of 1.5 years of monitoring. It is suggested to train at least twice as many panelists than are required. In this study, three times more assessors were trained. A good panel should provide results that are accurate, discriminating, and precise. Criteria for choosing an assessor for the "professional" panel was mostly taken from sensory standards DIN 10961 part 1, ISO 8586–2:2008 and Meilgaard et al. (2007). Additional criteria, such as willingness to cooperate and work in the panel for an extended period of time together with the availability of the person, were also taken into account. Sensitivity, motivation, ability to concentrate, and ability to recall and recognize odor qualities were also included as factors in the decision as suggested by Marin et al. (1988). Other factors, such as gender, age (general olfactory ability), presence of a respiratory disease (asthma, seasonal allergies, active colds), medication use, smoking, and occupational history, were noted, but were not discriminative (Dalton and Smeets, 2004).

Regular examination of the panel is carried out twice per month using reference mixture A and a new commercially available FMTM to train odor memory, to evaluate and keep the olfactory skills, and also to learn new odors. In this study, 7 months of monitoring is reported because monitoring and training is an ongoing process. The first 4 months (eight sessions in total) the panel analyzed reference mixture A, followed by 3 months (six sessions in total) analyzing FMTM. Vocabulary training for the FMTM analysis was carried out using pure standards diluted in ethanol. Results from regular training sessions are documented and the performance profile of each assessor is composed based on a statistical spreadsheet analysis using XLStat (panel analysis).

Sample Preparation and Instrumental Analysis

For the analysis of reference mixture A, 0.01 ml of the mix was injected into a 20 ml headspace (HS) vial that contained a 1-cm glass covered magnetic stir bar. Original FMTM was diluted in ethanol (1 μl/ml concentration). 0.01 ml of the diluted mix was injected into a 20 ml HS vial containing a stir bar. For kvass samples, 0.5 ml or 4 ml of the sample was measured into a 20 ml HS vial that also contained a 1-cm stir bar.

For sample preparation and injection, a CTC CombiPAL auto-sampler (Chromtech, Germany) with SPME option was used. The incubation time was 5 min at 60 °C, after which a 2-cm SPME fiber (50/30-μm DVB/Car/PDMS Stableflex, Supelco, Bellefonte, PA) was injected into the vial for 20 min at 60 °C for extraction. Samples were mixed in a single magnetic mixer (SMM, Chromtech) at 250 rpm. Volatiles were desorbed in an Agilent 7890 gas chromatograph equipped with a flame ionization detector (FID) and a sniffing port ODP-3 (Gerstel, Germany). The column effluent was split 1:1 between the FID and the sniffing port using deactivated fused silica capillaries (1-m length, 0.15-mm i.d.). The sniffing port was supplied with humidified air at 30 ml/min. The transfer line temperature was 300 °C. A capillary column DB-5MS (30-m length, 0.25-mm i.d. and 1-μl film thickness; J&W Scientific, Folsom, CA) was used in the GC. Helium gas (purity 5.0, AGA Eesti, Estonia) was used as a carrier at a constant flow of 2 ml/min. Splitless mode was used in a split/splitless injector at

250 °C. The initial oven temperature was 35 °C followed by a rate of 45 °C/min to 85 °C, then by 9 °C/min to 200 °C and then by 45 °C/min to 280 °C and held for 1 min (total run time 16.6 min).

FID responses confirmed the consistency of the injections and sample preparation for both replicates and dilutions. Intensity measurement was performed with the Gerstel ODP recorder program. If a panelist recognizes an odor he activates a microphone by pushing the specific remote control button for intensities 1–5 and describes the odor by quality. If intensities were not of interest, then button 3 with intensity 3 was chosen in all samples for registering the detection of an odor.

Statistical Evaluation

Panel average scores (number of positive detections as well as descriptions) were calculated for standard compound sniffing and GC–O analysis of the standard mixture. The scores were subjected to Kruskal–Wallis analysis ($\alpha = 0.05$), and the panel monitoring results were subjected to panel analysis ($\alpha = 0.05$) using XLStat.

Results and Discussion

Preliminary Analysis of Kvass

All assessors ($n = 29$) taking part in the training were subjected to pretesting of kvass, where only limited instructions were given to the assessors. Only 18 assessors were able to describe an odor at least one time, the others only registered compound detections. The vocabulary learned during the training was used only 15 times ($n = 29$; Table 2) compared to 137 times after training ($n = 20$, because the DF group with nine assessors did not describe the odor).

Table 2 Correct Assignment of Descriptions for Odor-active Compounds in Kvass before and After Training

Odor impression	Pretraining ($n = 29$)	After training ($n = 20$)
Cabbage	–	12
Butter	–	11
Grass	3	13
Whiskey	–	20
Banana	–	15
Potato	1	24
Mushroom	6	22
Almond	1	7
Kvass	4	13

Results of the Training Stage with Standard Compounds and Reference Mixture A

Nine previously memorized pure compounds were presented to the panelists on paper strips; the test results are given in Table 3. Assessors were divided into groups according to their different backgrounds. Assessors with a food technology background performed better than the other two groups and recognized, on average, 8.1±1.50 compounds out of nine correctly. However, a Kruskall–Wallis test indicated no significant difference between assessors with different backgrounds.

Data representing GC–O sniffing of reference mixture A are also presented in Table 3 (13 compounds in total, including impurities). As with the pure compounds, assessors with a food technology background had the best scores for both detecting and recognizing the compounds, 9.5±2.12 and 8.0±2.65 out of 13, respectively. A Kruskal–Wallis test indicated that there were again no significant differences between assessors with different backgrounds.

The most difficult compounds for the assessors to detect and recognize with GC–O were rotten cabbage (dimethylsulfide), grass (hexanal), and mushroom (1-octen-3-ol and octanol), mostly due to their higher odor thresholds (hexanal and 1-octen-3-ol) and low concentrations in the mix (dimethylsulfide and octanol were impurities).

GC–O Results of Kvass Samples

For sniffing the real product (kvass), three common olfaction methods were chosen: (1) DF, (2) AEDA, and (3) PI.

Detection Frequency

The DF group (Group 1) had ten sniffers ($n = 10$) that were able to detect an odor compound in kvass. The data for this assessment are tabulated and plotted as NIF values (Fig. 1a) and OID values (Fig. 1b). In Fig. 1a, 18 key compounds

Table 3 Average Correct Answers (±stdev) of Testing Nine Pure Compounds and Sniffing Reference Mixture A using GC–O

	Sensory assessors	Food technologists	Consumers
No. of assessors in a group	10	10	8
Correct answers of pure standards per person (max. of 9)	7.0±1.29	8.1±1.50	7.6±1.55
Correct detections of reference mix per person (max. of 13)	9.0±2.18	9.5±2.12	9.3±2.06
Correct recognitions of reference mix per person (max. of 13)	6.1±1.96	8.0±2.65	7.7±1.98

Assessors are broken down into the three types of panelists (consumers, food technologists, and sensory assessors)

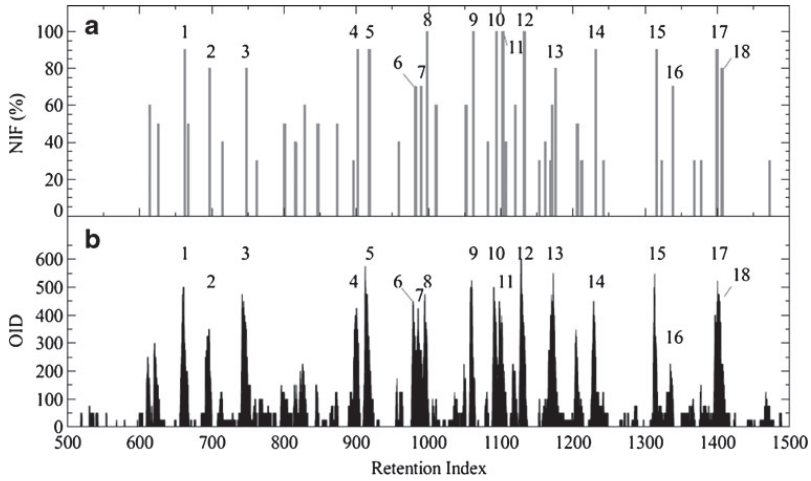

Fig. 1 **a, b** Comparison of detection frequency results by manual interpretation using a spreadsheet and a detection frequency "olfactogram" generated by the SNIFF program in Chemstation. 18 key compounds of kvass are numbered for better comparison

exceeding an NIF value of 60 % are numbered. The same numbers are used for the same compounds in Fig. 1b to allow for direct comparison between tables. Although the peak heights do not exactly coincide, the results are comparable with both spreadsheet and automatic data analysis (excepting perhaps compound 18 which was not distinguished using the SNIFF aromagram). To simplify data analysis, a SNIFF program can be used.

Aroma Extract Dilution Analysis

In the AEDA analysis carried out by Group 2 ($n=10$), samples were evaluated by the panelists in increasing dilution order. The impact of an odor-active compound is given by its FD in Fig. 2a and OID value in Fig 2b. Interpretation of AEDA data was challenging because gaps existed during sniffing of the dilution series. For example, occasionally, an assessor detected a compound in the most concentrated dilution, missed it in a second dilution, and detected it again in the third and/or fourth dilution. The error caused by the gaps should be minimized by using the equation suggested by Debonneville et al. (2002) and implemented in the SNIFF program. However, applying this correction is only possible in the case of a highly trained panel. For example, three peaks in Fig. 2b—butter (ret. index 1230), unknown (ret. index 1270), and butter (ret. index 1315)—were all detected once by one assessor in the most diluted sample. This resulted in high peaks in the aromagram and referred to an important compound, although this was probably caused by a beginner assessor not able to differentiate between background noise and actual odorous compounds. With the SNIFF program, using a signal-to-noise level of two to exclude such errors was impossible, and therefore, results were not very comparable with spreadsheet data handling.

PI Results

The PI group (Group 3; $n=9$) evaluated the odor impression and rated the intensity of a compound. Results of the spreadsheet calculation are shown in Fig. 3a, while Fig. 3b presents the results calculated using the SNIFF program. In total, 115 different compounds were detected by the panelists. In case of using a signal-to-noise level of two, the number of compounds detected was 48. Because the spreadsheet data coincide well with the SNIFF data, the only advantage is the automating data analysis using the program.

GC-O Method Comparison

Three different GC–O methods were tested to assess their applicability for odor analysis with an inexperienced panel and to study the data-handling complexity of each method. We found advantages and disadvantages to each method. DF did not provide any data on odor description. Some authors like Pollien et al. (1997) suggest the use of DF such that each assessor records the duration of the peak as well as the odor description. In this study, our inexperienced panelists did not judge the odor impression, because recording the exact ending of the odor (for SNIF data) may have been compromised while performing additional tasks simultaneously. Speaking at the same time when the assessor ought to be breathing out may impair their ability to sense the next odor to exit the GC–O. In addition, the thought process of deciding on the odor description often takes more time than the odor lasts. Furthermore, our

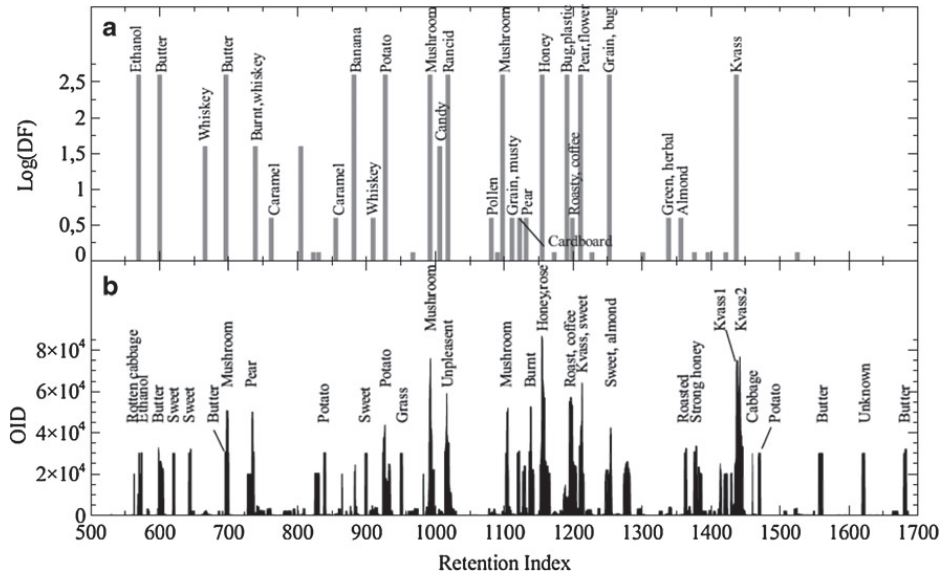

Fig. 2 **a, b** Comparison of AEDA results by manual interpretation using a spreadsheet and an AEDA "olfactogram" generated by the SNIFF program in Chemstation. For better comparison, the odor qualities have been added

recorder works only if the button is being pressed. It happens that the assessor often needs to release the button before they are finished speaking. On the other hand, if the objective is not to acquire SNIF data, this complementary method can be easily used.

Because the NIF method is unable to ascertain odor intensity, two compounds of unequal intensity would be viewed as being equal by this method (Reineccius and Vickers, 2004). Odors that are barely over the sensory threshold for all sniffers are treated equally as odors well above the sensory threshold for all sniffers. This can be avoided while applying a SNIFF program. For example, peaks 8, 9, 10, 11, and 12 were detected by all sniffers (Fig. 1a), but when using a SNIFF program, the intensities were distinguished more clearly. On the other hand,

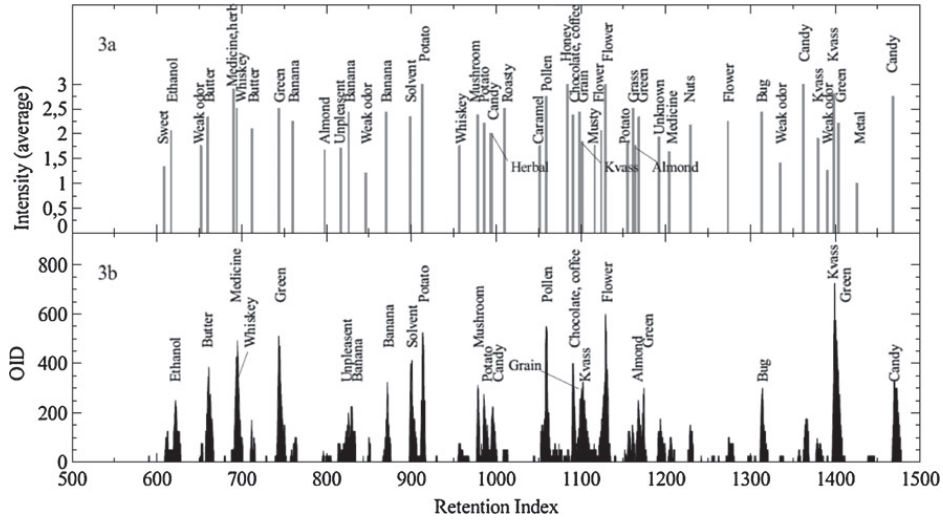

Fig. 3 **a, b** Comparison of posterior intensity results by manual interpretation using a spreadsheet (averaged) and a posterior intensity "olfactogram" generated by the SNIFF program in Chemstation. For better comparison the odor qualities are added

while using SNIF data analysis (where peak areas and not heights are used for key compound estimation), partially co-eluting compounds may be overestimated due to broader peak width, and early eluting compounds with narrow peaks may be underestimated (Delahunty et al., 2006).

AEDA is criticized for its incorrect assumption that intensity increases with concentration equally for all compounds (Audouin et al., 2001) and is not recommended to use while determining key odor-active compounds. Also, incompatibility with the conventional sensory measurements of odor intensity has been reported for dilution methods (Audouin et al., 2001). Dilution methods are also more time-consuming compared to other methods.

As a result of this study, the "professional" panel will continue training with the PI method because it provides enough information on the odorous compounds and proved to be feasible for a newly trained panel. Our panel is using a five-point scale, which is adequate; however, if possible, a larger scale should be used to better discriminate between odor-active compounds.

Concerning the key odor-active compounds in kvass, the PI and DF results were rather comparable, while the AEDA results differed to some extent giving attributing high values to rancid and bug-like compounds not distinguished in the first two methods. It was not in the scope of this study to identify odor-active compounds in kvass, but the odor qualities of key compounds found using all three methods appeared

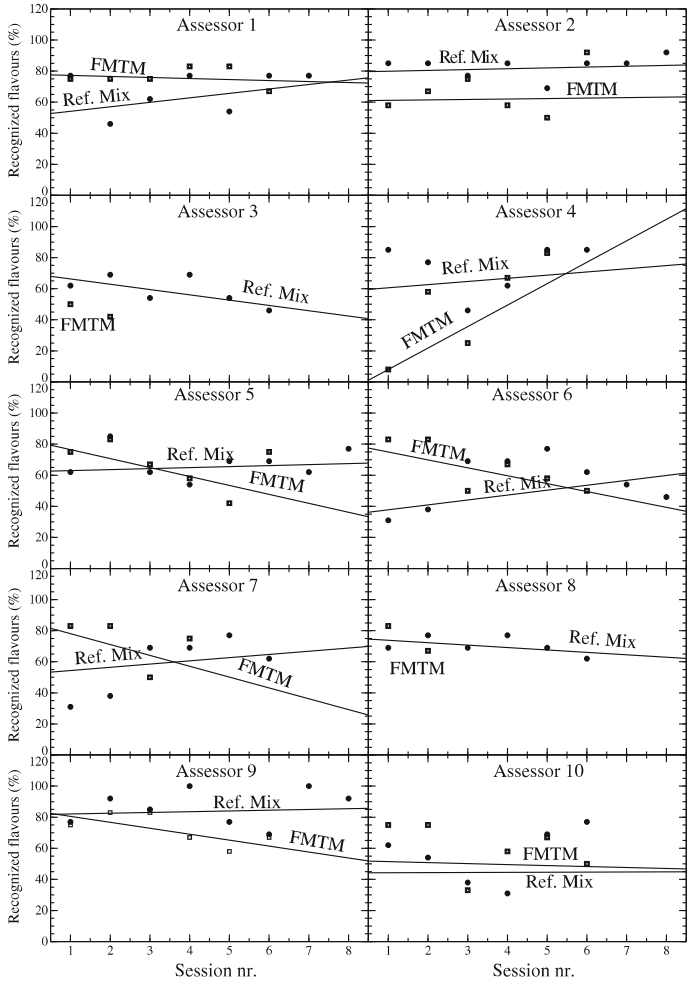

Fig. 4 Panel monitoring results. Percentage of correctly recognized compounds in reference mix A and fragrance materials test mix (*FMTM*) and the tendency for each assessor of the "professional" panel

to be butter, green, solvent, potato, mushroom, pollen, honey, chocolate/coffee, flower/pear, and kvass.

Monitoring of the "Professional" Panel

After training, about 80 % of the assessors were interested in becoming professional GC–O assessor. Ten assessors were chosen for further training according to their good detection and recognition skills. Nine female assessors were chosen together with one male.

All assessors selected had to perform GC–O analysis twice per month. Their performance profiles for reference mixture A and the FMTM are presented in Fig. 4, where the percentage of correctly detected compounds and the tendency of each assessor are provided. For example, assessor 9 is able to detect and identify most of the compounds in reference mixture A, but still requires more training with FMTM. Assessors 1 and 2 performed fairly well on both mixtures. Assessors 3 and 8 are showing signs of fatigue and a drop in motivation, because the tendencies for reference mixture A drop off with time and they missed several training sessions for the FMTM. Figure 5 shows which compounds in reference mixture A or FMTM were difficult to detect and/or recognize for each assessor so he can further refine their abilities using pure standards. This graph was given to each assessor, but only assessors 1 and 2 have are reproduced here. Assessor 1 never detects *grass* (hexanal) in reference mixture A and has problems with detecting *urine* (benzoic acid) in FMTM. In Fig. 5, assessor 2 recognizes all compounds near or above the panel average level. In addition, the information in Fig. 5 provides valuable information on how each assessor is using the scale.

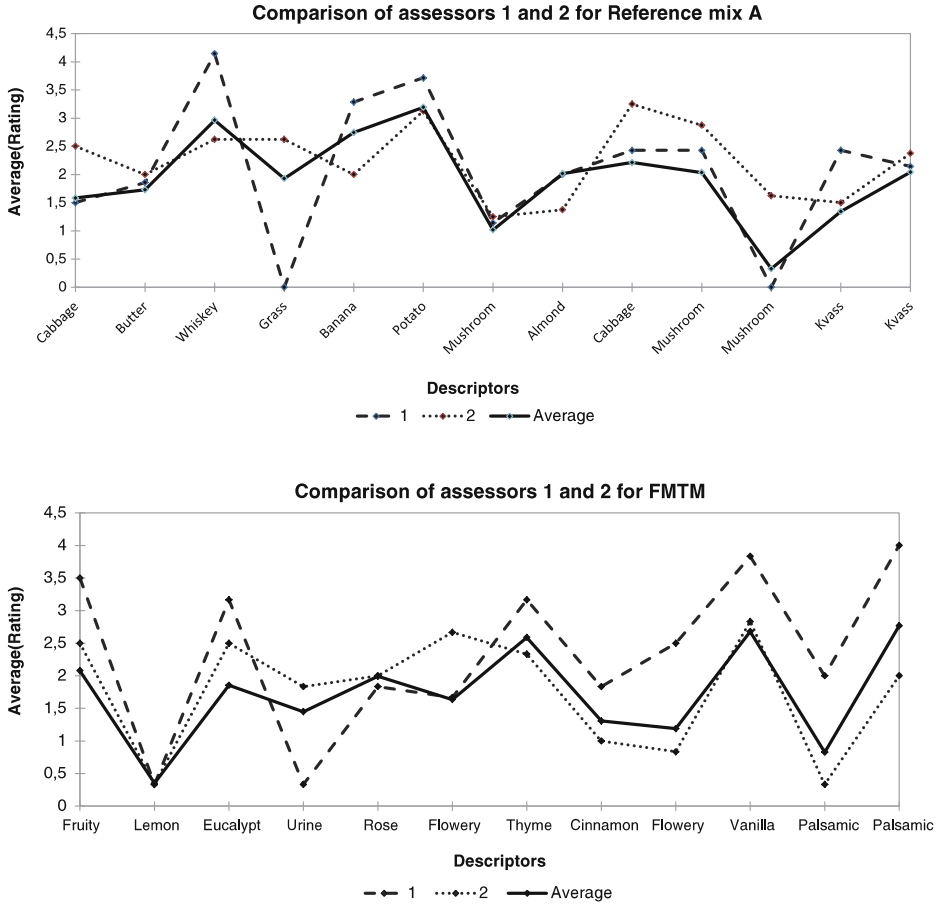

Fig. 5 Comparison of "professional" assessor 1 and 2 to the panel average for the analysis of reference mixture A and the fragrance materials test mix (*FMTM*). All sessions were averaged for each mix

The Effect of Training

The effect of training was also evaluated by the trainees themselves. The theoretical part was found to be helpful for some; all the other stages were either important or very important. It was agreed that steps 2 and 4, learning the vocabulary and sniffing kvass, respectively, were most effective. The abilities of assessors were often found to be influenced by their state of health, mostly the common cold in wintertime. All agreed on the importance of the training, but suggested that it should be carried out over a shorter period (less than 4 months) and could be more intensive. Also, more standard compounds could be used to enlarge the vocabulary for kvass analysis. In this case, more training with reference mixtures and more feedback would have been beneficial.

Conclusion

The training method developed in this work is suitable to train assessors to perform complex GC–O tasks. The ability to detect and recognize odor-active compounds in kvass improved dramatically. Interestingly, the specific background of the panelists was not found to influence their ability to perform GC–O tasks. The PI method was found to be most feasible for a newly trained GC–O panel, and the SNIFF program eased the data-handling process. This method is also the least time-consuming of the three studied. Custom statistical tools were developed for professional panel monitoring which provide a good overview of the skills and potential of each assessor. These tools were also found to aid in the selection process for additional GC–O training.

Acknowledgments The authors wish to thank all the volunteers who participated in the study. The financial support for this research was provided by the Enterprise Estonia project EU29994.

Conflict of interest None

Compliance with Ethics Requirements

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PUBLICATION II

Seisonen, S., Kivima, E., Vene, K.

Characterization of the aroma profile of different honeys and corresponding flowers using SPME-GC/MS and GC-Olfactometry.

Food Chemistry,169, 34–40, 2015.

Food Chemistry 169 (2015) 34–40

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Characterisation of the aroma profiles of different honeys and corresponding flowers using solid-phase microextraction and gas chromatography–mass spectrometry/olfactometry

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ARTICLE INFO

Article history:
Received 27 March 2014
Received in revised form 27 July 2014
Accepted 29 July 2014
Available online 5 August 2014

Keywords:
Honey
GC–O
SPME
GC–MS
Flavour
Calluna vulgaris
Rubus idaeus
Brassica napus
Frangula alnus

ABSTRACT

The aroma profiles of thirteen different honey samples from four botanical origins: heather (*Calluna vulgaris*), raspberry (*Rubus idaeus*), rape (*Brassica napus*), alder buckthorn (*Frangula alnus*) and the blossoms of the four corresponding flowers were investigated to find odour-active compounds exclusively representing specific honeys based on odour-active compounds from the blossoms. Gas-chromatography–mass spectrometry (GC–MS) and gas-chromatography–olfactometry were used to determine and identify the odour-active compounds. Data was analysed using agglomerative hierarchical clustering and correspondence analysis. Honeys from the same botanical origin clustered together; however, none of the identified compounds were exclusive to a particular honey/blossom combination. Heather honey had the flavour profile most different to the others. Isophorone and 2-methylbutyric acid were found only in heather honeys. Heather honey was characterised by having more "sweet" and "candy-like" notes, raspberry honeys had more "green" notes, while alder buckthorn had more "honey" and "floral" notes.

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1. Introduction

Honey is a highly regarded food product in all parts of the world. The main parameters of honey quality, which also influence its price, are derived from its botanical origin. Several articles have been published on marker compounds from the volatile fraction, which could be used to identify the floral origin (Castro-Vazquez, Diaz-Maroto, Gonzalez-Vinas, & Perez-Coello, 2009; Castro-Vazquez, Diaz-Maroto, & Perez-Coello, 2007; de la Fuente, Sanz, Martinez-Castro, Sanz, & Ruiz-Matute, 2007; Guyot, Bouseta, Scheirman, & Collin, 1998; Guyot, Scheirman, & Collin, 1999; Jerković, Tuberoso, Marijanović, Jelić, & Kasum, 2009; Piasenzotto, Gracco, & Conte, 2003). Instrumental analysis has also been combined with descriptive sensory analysis, where, for example, heather honey was described with attributes "ripe fruit", "spicy", "woody" and "resin" (Castro-Vazquez et al., 2009). Cuevas-Glory, Pino, Santiago, and Sauri-Duch (2007) reviewed volatile analytical methods for determining the botanical origin of honey, pointing out extraction methods, fibres and extraction conditions used.

Solid-phase microextraction (SPME) as an aroma extraction method eliminates the use of (toxic) organic solvents, allows the quantification of a large number of molecules, requires little or no manipulation/preparation of samples, substantially shortens the time of analysis and, moreover, it is simple (Pontes, Marques, & Cámara, 2007). SPME has been widely used in analysis of different food products including honey (Piasenzotto et al., 2003; Plutowska, Chmiel, Dymerski, & Wardencki, 2011; Wolski, Tambor, Rybak-Chmielewska, & Kędzia, 2006).

SPME sampling can be performed in three basic modes: direct extraction, headspace extraction (HS) and extraction with membrane protection. The main advantage of the HS analysis is that it is carried out on an untreated sample (Piasenzotto et al., 2003) and the profile of the isolated volatiles is closely associated with sensory perception (Kaškoniene, Venskutonis, & Čeksteryte, 2008).

Heather honey has been previously characterised by a relatively high content of phenolic compounds, such as guaiacol, p-anisaldehyde and propylanisole (Castro-Vazquez et al., 2009). Phenylacetic acid was found exclusively in *Calluna vulgaris*

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http://dx.doi.org/10.1016/j.foodchem.2014.07.125
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(heather) honey (Guyot et al., 1999). Radovic et al. (2001) analysed 43 authentic honey samples of different botanical and geographical origins by means of dynamic headspace GC–MS, in order to assess marker compounds (if/when existing) of both botanical and geographical origin. Honey samples were of nine different botanical origins (seven acacia, nine chestnut, three eucalyptus, eight heather, two lavender, four lime, four rape, two rosemary and four sunflower) and from eight different countries (one from Denmark, ten from Germany, thirteen from Italy, eight from France, four from The Netherlands, two from Spain, two from Portugal and three from England). Radovic et al. (2001) identified phenylacetaldehyde as a characteristic compound to heather honeys.

According to Radovic et al. (2001) the authenticity of rape honeys could be confirmed by the absence of 2-methyl-1-propanol; however, this compound was absent also in one of the seven acacia honeys analysed, therefore it was emphasised that the simultaneous presence of dimethyl disulphide is necessary in order to confirm the authenticity of rape honeys. Plutowska et al. (2011) determined volatiles from popular Polish honeys (rape, acacia, linden, buckwheat, heather, polyfloral and honey-dew) by HS–SPME and found that the presence of dimethyl disulphide is not a peculiar feature of rape honey and can also be found in other honeys. Authors also emphasised that a much more significant feature to rape honeys is the lack or much lower concentrations of characteristic volatile compounds occurring in other honeys, e.g., linalool oxides, furfural and phenylacetaldehyde, which were present in most honey samples of different botanical origins. Kaškoniene et al. (2008) also found in their study that dimethyl disulphide was present only in six rape honeys out of eleven, while 2-methyl-1-propanol was absent in all of them.

Raspberry honey is characterised by the presence of 2-ethenyl-2-butenal, 3-methylhexane, 3-methylnonane, 3-pyridinemethanol, β-myrcene, cyclopentanemethanol, norbornane, and undecanal (Špánik et al., 2013), while tere is no literature available on volatile fraction of alder buckthorn honey.

Not all volatile compounds have significant impact on honey aroma due to different odour thresholds and interactions between compounds. GC–olfactometry (GC–O) can be used to select key odour-active compounds affecting the aroma of the honey. There is very limited information available about GC–O analysis of honey. Pino (2012) carried out a study on black mangrove honey using aroma extraction dilution analysis (AEDA) complemented by quantitative analysis and calculation of odour activity values. It was concluded that (*E*)-β-damascenone, nonanal and decanal are primarily responsible for the distinctive and characteristic aroma of black mangrove honey.

Alissandrakis, Tarantilis, Pappas, and Pashalis (2011) and Amtmann (2010) have conducted studies on volatile compounds present in honey and flower using GC–MS. It was found that relatively high percent of volatile compounds were overlapping in flowers and honeys, which allowed on floral markers to be proposed. However, since many of the compounds were common in the plant kingdom, they were present in various plants and honeys.

The aim of the present study was to determine floral markers influencing the aroma profile of honeys from different botanical origins by using HS–SPME–GC–O. Additionally, blossoms from representing plants were studied to find odour-active compounds that are carried over from the blossom to the honey. To the authors' best knowledge, GC–O has not been used on this purpose before.

2. Materials and methods

2.1. Materials

Honey samples were collected from local beekeepers in Estonia. Thirteen different honey samples were analysed. Samples 1 and 2 were unifloral raspberry honeys, 3–5 unifloral rape honeys, 6–8 honeys with high rape pollen content, 9–10 unifloral heather honeys, 11 honey with high heather pollen content and 12–13 honeys with high alder buckthorn pollen content. Samples 12–13 could also be unifloral honeys, but there is no literature available determining the content of pollen of alder buckthorn in unifloral honey. Visually samples 12 and 13 were rather different from other samples because of their dark colour and liquid consistency. Honey samples were stored at 4 °C until analysis. Plant blossoms were chosen according to the honey pollen analysis and harvested at the time of blossoming.

2.2. Melissopalynological analysis

Melissopalynological analysis was carried out according to the non-acetolytic method described by Louveaux, Maurizio, and Vorwohl (1978). The pollen counts were expressed as percentages after counting 500–600 pollen grains (Table 1). The identification of the pollen types were based mainly on the reference collection of the department of Food Processing in Tallinn University of Technology and data provided by Ricciardelli ÓAlbore (1997). An Olympus CX21 (Japan) binocular light microscope with 40 x 15 magnification was used. Required pollen contents to consider honeys unifloral can be found from previous research carried out by Kivima et al. (2014).

Table 1
The main pollen types of honey samples (%). Percentages in boldface refer to unifloral honeys; the plus sign (+) stands for minor pollen (<1%).

Pollen type	Honey samples												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Cruciferae													
Brassica napus s.l.	17	11	**60**	**77**	**76**	51	50	43	40	27	9	+	23
Ericaceae	1										3		
Calluna vulgaris									**16**	**27**	4		
Leguminosae													
Melilotus officinalis s.l., *Trifolium repens* s.l.	1	4	5	10	2	5	10	4	18	28	3	21	1
Trifolium pratense s.l.	+		1	5	+	+	5	+	3	4	19	1	+
Rhamnaceae													
Frangula alnus			1		2				2	3	1	**42**	**22**
Rosaceae													
Rubus idaeus s.l.	**67**	**79**	17	2	8	7	17	14	6	5	31	33	32
Salicaceae													
Salix spp.	6	5	9	+	6	27	7	34	1	1	14	+	5

2.3. Chemicals

Pure standards (GC grade) of furfural, eugenol, (*E*)-β-damascenone, vanillin, linalool, methional, furaneol and acetone were purchased from Merck (Darmstadt, Germany). Phenylacetaldehyde, benzoic acid, hexane, ethyl acetate, methylene chloride and NaCl were from Sigma–Aldrich (St. Louis, MO). Ethanol (96.6%) was acquired from Rakvere Piiritustehas (Rakvere, Estonia). Kovats retention indices were determined using a C_8–C_{22} mix from Sigma–Aldrich.

2.4. Sample preparation for solid-phase microextraction (SPME)

50% w/w dilution with water was made for all honey samples. Diluted honey (1 mL) and 1 g of NaCl were measured into a 20-mL SPME vial with a glass covered stirrer. Blossoms were placed into 20-mL SPME vial immediately after harvesting depending on the size of the blossoms, covering approximately 1 cm above the bottom of the vial. In order to apply the same headspace volume to all the samples and to avoid cutting the flowers, volume was used instead of weight for the samples. Two replications of each sample were done for GC–O for each assessor (three assessors in total) and one sample for GC–MS. All vials were capped with PTFE-silicon septa and placed in an autosampler tray at room temperature. Samples were brought one-by-one into magnetic stirring chamber for volatile extraction using a method described in the next section. Magnetic stirring was used for honey samples but not for blossoms. After the extraction process the fibre was injected into the GC inlet for desorption for 10 min (either GC–MS (TOF) or GC–O), followed by the oven temperature program described in the next section.

2.5. Parameters for GC–MS and GC–O

For SPME, 30/50 μm DVB/Car/PDMS Stableflex 2-cm long fibre from Supelco (Bellefonte, PA) was used. The GC column for both GC–MS (Agilent 6890; Agilent Technologies, Santa Clara, CA) and GC–O (Agilent 7890) was a DB5-MS (30 m × 0.25 mm × 1.0 μm; Agilent). The GC–MS inlet was a PTV, while the GC–O inlet was split/splitless using a Merlin Microseal (Agilent), and both were run in splitless mode. A 0.75 mm i.d. liner at 250 °C was used in both injectors. Carrier gas was He, 1.0 mL/min for GC–MS and 2.0 mL/min for GC–O. The GC–MS was equipped with a time-of-flight detector (Waters, Manchester, UK) and the GC–O was equipped with a flame ionisation detector (Agilent) and odour detection port (Gerstel, Mülheim an der Ruhr, Germany). For GC–MS data analysis the NIST05 library was used. The oven temperature programme for best separation of volatiles, and SPME extraction time and temperature for best sensitivity were previously optimised. An incubation time of 5 min at 60 °C for honey and 35 °C for blossoms with an extraction time of 20 min (250 rpm) and desorption time 10 min were chosen as optimum. The oven program for both GC–MS and GC–O was from 35 °C, 45 °C/min to 85 °C, 9 °C/min to 200 °C, 45 °C/min to 280 °C holding time 1 min (total 16.67 min). For identification of the odour-active compounds, the results of GC–MS and GC–O were correlated using Kovats retention indices.

2.6. Statistical analysis

For GC–O data, detection frequency method was used and results were inverted into percent values. Detection frequency method estimates the odour intensity based on recording detected odours from a number of sniffers. More than 33% was counted as a signal, meaning the odour was detected at least two times out of six analyses. Odour descriptions were generated by assessors. Compounds with similar odour descriptions were summed for statistical analysis. Mapping of samples and flavour descriptions was carried out using correspondence analysis (CA) (XLStat, Addinsoft, New York, NY). Correlations between attributes were found using Pearson correlation coefficient ($p = 0.05$). Agglomerative hierarchical clustering (XLStat) based on dissimilarities was used to explain the results based on clustering.

3. Results and discussion

3.1. Odour-active compounds

Forty-six odour-active compounds which had detection frequency more than 33% were found using GC–O. Compounds were extracted by a DVB/CAR/PDMS fibre that was selected according to research carried out by Plutowska et al. (2011) and which showed the best efficiency and repeatability. Table 2 shows the presence of each compound according to GC–O data. Compared to blossoms more odour-active compounds were detected in honey samples and the odours were generally more intense. GC–MS data were used for the tentative identification of odour compounds detected by GC–O assessors. For GC–MS data analysis, Kovats retention indices and standard compounds were used. Due to co-elution, where higher intensity compounds were masking some low intensity compounds, we could have missed some odour-active compounds. Moreover, according to Table 2, the absence of a specific compound means that the compound was not detected using GC–O; it still might occur in the sample, but below the odour threshold value.

The compounds present in all the honey samples were butyric acid (cheesy), methional (potato), oct-1-en-3-one (mushroom), camphene (camphor), phenylacetaldehyde (honey), 2-hydroxybenzaldehyde (medicinal), (*Z*)-linalool oxide (floral), 3,5-dimethyl-2-ethylpyrazine (coffee), (*E*,*Z*)-2,6-nonadienal (green), benzoic acid (urine), phenylacetic acid (honey), carvone (green), hydrocinnamic acid (floral), hexyl hexanoate (apple), (*E*)-β-damascenone (apple), vanillin (vanilla) and δ-decalactone (coconut). Eugenol (clove) and geranyl acetone (floral) were present in most of the samples.

Guyot et al. (1999) investigated marker compounds of heather honeys by isolating aroma compounds by extraction with dichloromethane, followed by a Likens–Nickerson steam distillation/solvent extraction. They suggested *p*-anisaldehyde as a marker compound for heather honeys. In this study *p*-anisaldehyde was also detected in Estonian heather samples, but it was present also in all the other investigated honey samples. Guyot et al. (1999) also found that the presence of benzoic acid and isophorone indicated floral origin within the heather family; this corresponded well with the current study, where these compounds played an important role in heather honey aroma profile according to GC–O results. In this study isophorone was exclusively found in heather honeys and benzoic acid was present in all of the heather honey samples. 2-Methylbutyric acid was found exclusively in heather honey. Additionally, linalool was absent in both heather honey and heather blossom; it was also not detected in heather honey by Castro-Vazquez et al. (2009) and Wolski et al. (2006). Castro-Vazquez et al. (2009) used extraction with dichloromethane followed by simultaneous distillation–extraction, while Wolski et al. (2006) used SPME for isolation of the aroma compounds.

Robertson, Griffiths, Woodford, and Birch (1995) investigated volatiles at various stages of inflorescence development, bud formation, flowering, fruit formation and ripening of a red raspberry. The samples were entrained on the porous polymer Tenax TA and analysed by thermal desorption-GC–MS. Robertson et al. (1995) found (*Z*)-3-hexenyl acetate and *E*-β-ocimene to be major volatile compounds in raspberry flower. In our research we could not detect these compounds with GC–O, which could be explained

Table 2
Compounds and their detection frequencies according to GC–O (1–2 raspberry honeys, 3–8 rape honeys, 9–11 heather honeys, 12–13 alder buckthorn honeys; Ras – raspberry, Al – alder buckthorn, Rap – rape, He – heather).

Compound	Kovats RI[a]	Observed RI	Ident.[b]	Fl. Description	1	2	3	4	5	6	7	8	9	10	11	12	13	Ras	Al	Rap	He
Unknown		808		Potato															66		
Butyric acid	820	808	L,	Cheesy	100	100	100	100	100	100	100	100	83	66	83	83	83	33			
Furfural	829	835	L, St	Sweet				33	33	33		33	33	33	33				66		
(*E*)-2-hexenal	844	844	L	Grass														33	100		66
2-Methylbutyric acid	873	866	MS, L	Potato chips									33	50	33						
Methional	909	904	St, L	Potato	33	33	66	33	83	33	33	66	33	50	50	83	50	50	66	50	33
2-Acetylfuran	893	912	MS, L	Candy									33	33	33						
Unknown		912		Whiskey	83	66	66	66	100	100	100	100					66		66	66	
Unknown		944		Lemonade	33					33			33	33	33		33				
1-Octen-3-one	976	968	MS, L	Mushroom	100	100	33	66	83	83	83	83	66	66	83	100	50	100	100	50	66
Camphene	953	972	MS, L	Camphor	33	33	33	66	50	33	50	66	33	33	33	50	50	100	50	66	66
Dimethyl trisulphide	974	982	MS, L	Sulphur	100	66	66	100	100	66	100	100	83	66	83					100	
2-Ethyl-5-methylpyrazine	993	991	L	Sweet														50	83		66
2-Ethyl-1-hexanol	1032	1044	L	Grass																100	
Phenylacetaldehyde	1049	1046	St, MS, L	Honey	100	100	83	100	100	83	66	100	100	100	100	100	66		50		
2-Hydroxybenzaldehyde	1041[p]	1064	MS, L	Medicinal	33	33	33	33	33	33	33	33	33	33	33	66	33				
Furaneol	1064	1071	St, L,	Caramel, honey					66	50	33	33	66	33	33	33	33				
Acetophenone	1065[p]	1081	MS, L	Green	66	33	33				33	33	66	50	66			50	83		33
(*Z*)-linalool oxide	1070	1088	MS, L	Floral	66	83	50	83	83	66	66	66	50	50	66	83	66	33	83		50
Linalool	1100	1092	L, St	Floral	50	66		33	50	33	33	100				50	50	83	50		
3,5-Dimethyl-2-ethylpyrazine	1083	1094	L	Coffee	66	66	50	83	83	66	33	50	50	50	66	83	83				
2-Phenylethylalcohol	1118	1118	MS, L	Floral	66	33	33	50	83	50	50		50	50	50	83	50		66		33
Isophorone	1118[p]	1126	MS, L	Candy									50	66	33						
Unknown		1129		Green	33																
(*E,Z*)-2,6-nonadienal	1154	1148	L	Green	50	50	50	33	83	33	50	33	33	33	33	33	33		66		
Octanoic acid	1179[p]	1156	L	Leather														83	83	66	50
Lilac aldehyde A	1155[p]	1169	MS, L	Honey	33		33		33				66	66	50	83	33				
Benzoic acid	1276[n]	1178	St, MS, L	Urine	33	33	50	66	50	66	66	66	33	50	66	33	33				
Ethyl benzoate	1185	1186	MS, L	Green	83	50	50	33	33	50	33	50	33	33					33		
Unknown		1198		Leather														50			
Phenylacetic acid	1262	1236	MS, L	Honey	66	66	83	66	83	66	100	100	66	66	66	100	50	66	66	33	33
Ethyl-2-phenylacetate	1244[p]	1246	MS, L	Honey		33							33	50							
DL-carvone	1253	1259	MS, L	Green	33	66	33	33	33	33	66	66	33	33	50	33	33				
p-Anisealdehyde	1252[p]	1273	MS, L,	Honey			33	66	33		50	33	33	50	33	33	33		66		
γ-Butyrolactone	1299	1288	L	Honey	50		33		33				50	33	33	100	33	66			
Hydrocinnamic acid	1321	1321	MS, L	Floral	100	100	83	100	100	83	100	100	83	83	83	100	83		33		33
Methyl 2-methoxybenzoate	1340[p]	1345	L	Honey	50	33	33	33			50	33	33		33						
Ethyl dihydrocinnamate	1351	1351	MS, L	Floral		33			66	33	33	50	33	50	33		33				
Eugenol	1364	1358	St, MS, L	Clove	66	66	50	50	33	33		66	66	33	33	83	50		66		50
Hexyl hexanoate	1379	1369	MS, L	Apple	83	100	33	33	33	33	33	50	83	66	66	33	33				
β-Damascenone	1386	1385	St, MS, L	Apple	66	66	50	83	66	50	100	100	50	50	50	100	83	66			
Vanillin	1410	1403	St, MS, L	Vanilla	66	83	33	66	83	50	66	66	83	66	50	83	50				
Geranyl acetone	1448	1444	MS, L	Floral	50	50	33	33	33	50	33	50	83	33	50			66	66		
δ-Decalactone	1469	1459	MS, L	Coconut	83	83	33	50	50	33	100	100	50	66	66	50	50	33	33	50	
Unknown		1505		Sweet	33	33							33	66	33		33				
Unknown		1536		Sweet	33	33															

[a] www.flavornet.org.
[p] www.pherobase.com.
[n] NIST 2.0.
[b] Identification based on MS – GC–MS, St – standard, L – literature, RI.

by the different methods used and the high odour threshold values of both compounds.

As literature about alder buckthorn honey is absent, any links with previous researches could not be made. Also, it was not possible to highlight any compounds specific to raspberry or to alder buckthorn honeys.

Rape honey has been characterised by the presence of dimethyl disulphide (Radovic et al., 2001), which was not found during this study. Instead there was dimethyl trisulphide found in rape blossoms and in all honey samples except for alder buckthorn.

3.2. Clustering of honey samples

The data matrix was subjected with odour descriptions to hierarchical cluster analysis (HCA) based on dissimilarities. As seen from the Fig. 1, honey samples from the same botanical origins have clustered together. Heather honeys have the most similar flavour profiles. Raspberry honeys have similarities with rape honeys, which could be explained by small amounts of raspberry pollen found in rape honeys. Heather honeys group together with alder buckthorn. Aliferis, Tarantilis, Harizanis, and Alissandrakis (2010) used HCA on GC–MS data and also obtained very good classification results of different honeys according to their botanical origin.

3.3. Aroma profiles

The aroma profiles of different honeys were rather similar. The most commonly used descriptors were floral and honey-like, and also green. Typical non-herbal aromas were leather, mushroom, metallic and urine. Many compounds also had sweet aromas, like candy and vanilla. Figs. 2 and 3 show the correlation of honey samples and blossoms with flavour characteristics from GC–O,

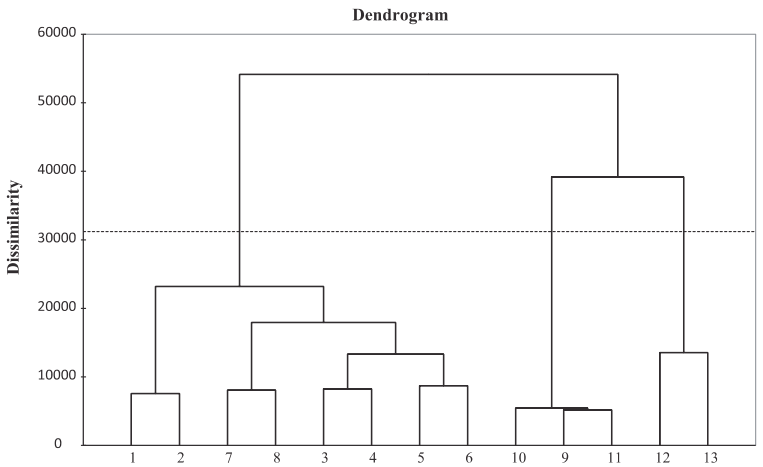

Fig. 1. Agglomerative hierarchical clustering (AHC) of 13 investigated honey samples; 1–2 raspberry honeys, 3–8 rape honeys, 9–11 heather honeys, 12–13 alder buckthorn honeys.

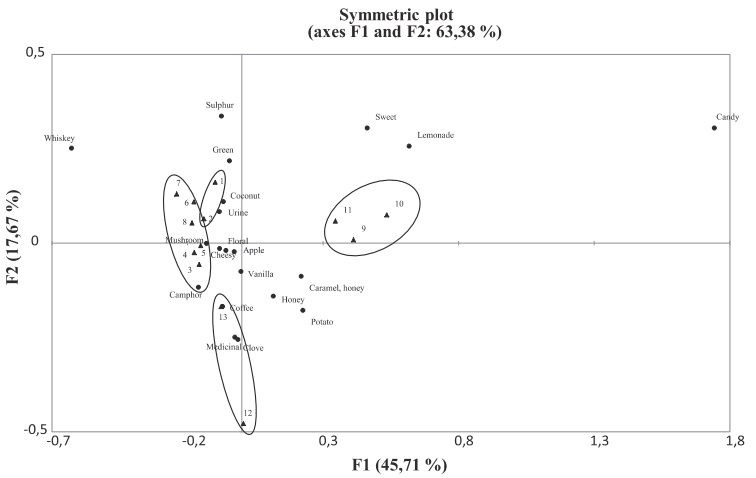

Fig. 2. Correspondence analysis of 13 honey samples (1–2 raspberry, 3–8 rape, 9–11 heather, 12–13 alder buckthorn) and flavour characteristics from GC–O.

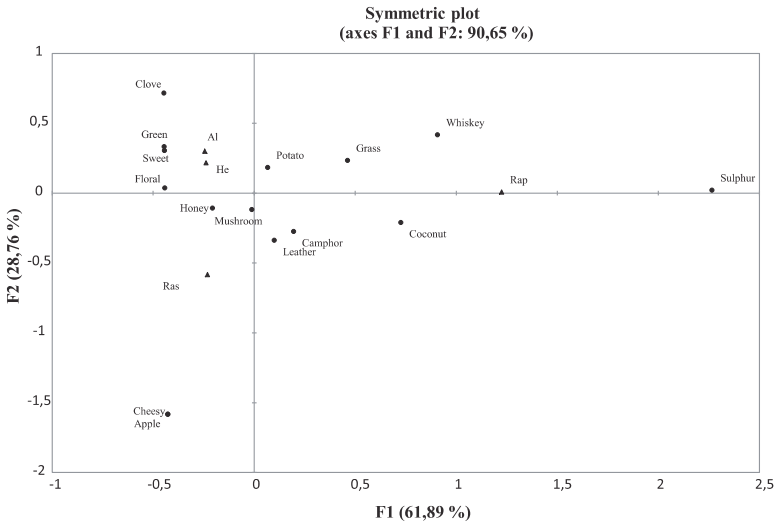

Fig. 3. Correspondence analysis of 4 blossoms (rape, heather, raspberry, alder buckthorn) and flavour characteristics from GC–O.

accordingly. Heather honeys had more odour-active compounds than other investigated samples and could be described as having more sweet candy-like aromas. Raspberry honey can be characterised by a larger number of green notes and lack of honey notes. Rape honey has the poorest aroma profile without many characteristic notes as also mentioned by Plutowska et al. (2011). The only important feature in rape honeys as well as blossom is sulphur content. Rape blossom seems to be the source for sulphur and all the samples contain rape pollen to some extent, which explains sulphur in the aroma profiles of most of the samples. Alder buckthorn honeys tend to have more floral and honey notes and less green and sweet/candy characteristics. Additionally, sulphur was not present (over threshold), unlike the other honey samples.

4. Conclusions

In terms of this research, no marker compounds were common to the honey and the corresponding blossom; no volatiles were found which are coming from a specific blossom to the specific honey. The most important compounds indicating the botanical origin of heather honeys are the presence of isophorone and 2-methylbutyric acid and the absence of linalool. Dimethyl trisulphide refers to the content of rape pollen in the honey. Flavour profiles of heather, rape, raspberry and alder buckthorn honeys are rather similar. There are some nuances in flavour composition and intensities which make the honeys from the same botanical origin cluster together. Heather honey has the biggest differences due to odour-active compounds which were not present in the other honeys. Heather honey can be characterised by having more "sweet" and "candy like" notes, raspberry honey "green" notes, alder buckthorn "honey" and "floral" notes and rape honey has the poorest profile, without any characteristic peaks.

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PUBLICATION III

Seisonen, S., Vene, K., Koppel, K.

The current practice in the application of chemometrics for correlation of sensory and gas chromatographic data.

Food Chemistry, 210 (1), 530−540, 2016.

Food Chemistry 210 (2016) 530–540

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Review

The current practice in the application of chemometrics for correlation of sensory and gas chromatographic data

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ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form 24 April 2016
Accepted 28 April 2016
Available online 29 April 2016

Keywords:
Chemometrics
Sensory-instrumental relations
PLSR
Gas chromatography

ABSTRACT

A lot of research has been conducted in correlating the sensory properties of food with different analytical measurements in recent years. Various statistical methods have been used in order to get the most reliable results and to create prediction models with high statistical performance. The current review summarises the latest practices in the field of correlating attributes from sensory analysis with volatile data obtained by gas chromatographic analysis. The review includes the origin of the data, different pre-processing and variable selection methods and finally statistical methods of analysis and validation. Partial least squares regression analysis appears as the most commonly used statistical method in the area. The main shortcomings were identified in the steps of pre-processing, variable selection and also validation of models that have not gained enough attention. As the association between volatiles and sensory perception is often nonlinear, future studies should test the application of different nonlinear techniques.

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http://dx.doi.org/10.1016/j.foodchem.2016.04.134
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1. Introduction

Investigating the relationship of the sensory perception of food with its volatile chemical components enhances the understanding of the flavour of any food. Although high correlation between volatile components and sensory attributes may not refer to a causal connection, it is indicating that the variables are changing in the same manner. For example if the sample contains a high level of a measured volatile component it may be an indication of a high intensity of a sensory attribute with which it is correlated (Owusu, Petersen, & Heimdal, 2013). Sensory data and instrumental measurements are related in a variety of contexts and this serves a number of objectives. The most common academic use is to investigate the mechanisms by which physical properties of foods and food products act to produce specific sensations during viewing, smelling or eating. This is also of interest to the food industry. Sometimes, the objective is to establish which sensory attributes can be accurately predicted by instruments, or a combination of instruments to improve online quality assurance (Macfie & Hedderley, 1993). Replacing sensory measurements with instrumental methods is needed as sensory methods may be expensive to implement, time consuming, and sometimes cannot be implemented on-line for immediate feedback (Chambers & Koppel, 2013). Sensory judgments are often less reliable because humans as detectors are subjective and are prone to physiological and psychological fluctuations. Therefore, the data collected may contain more noise compared to instrumental measurements. Nowadays, various statistical methods are used to correlate sensory and instrumental data and to create prediction models with high statistical performance. Macfie & Hedderley (1993) reported a review on methods used for correlating instrumental and sensory data. The authors emphasised the importance of different pre-processing techniques in order to get reliable results. At that time, the number of statistical techniques used tended to be higher than the techniques used today. For example the review covered techniques like simple correlation, multiple regression, principal component regression, partial least squares, canonical correlation and redundancy analysis. Recently Zielinski et al. (2014) reviewed and demonstrated the use of chemometrics in assessing different properties of fruit juices and summarised the overall features, advantages and disadvantages of different chemometric tools that could be applied to experimental data. It was emphasised that the use of chemometrics requires understanding of the method's principles, and the meaning of the individual input parameters as well as a critical evaluation of the obtained results.

The aim of the current review is to investigate recent practices when chemometrics is used to relate volatile data obtained by gas chromatography with sensory attributes. The main shortcomings of the methods and suggestions for future studies are given.

2. The origin of the raw data

2.1. Instrumental analysis

The experiments of measuring volatiles that potentially have an influence on sensory perception could be designed in various ways, resulting in different types of received data. Instrumental data of volatiles collected have been mainly based on gas chromatography-mass spectrometry (GC–MS), gas chromatography-olfactometry (GC-O), gas chromatography-flame ionisation detector (GC-FID) or by combining these techniques. GC-O is a direct approach used to determine which chromatographic peaks possess odour activity which is a big advantage in terms of correlating volatiles data to sensory analysis. Because GC-O uses humans as detectors, there are reliability issues when comparing to only instrumental analysis like GC–MS. In order to get reliable results, the number of assessors used should be as high as possible depending on the GC-O method used.

When performing GC-O analysis, dilution (Michishita et al., 2010), detection frequency (Bansleben et al., 2009), posterior intensity (Schulbach, Rouseff, & Sims, 2004; Thomsen et al., 2012) and also modified frequency (Campo, Ferreira, Escudero, & Cacho, 2005) techniques have been used. Aroma extract dilution analysis (AEDA) and combined hedonic aroma response measurement (Charm Analysis) are the dilution methods that are used most often. AEDA measures the highest sample dilution at which the odour of the analysed compound is still detectable. Charm Analysis, proposed by Acree and Barnard (1984), also records the duration of odours which is taken into account with the final dilution at which the compound is detected. Detection frequency methods measure the intensity of the compound by calculating the number of assessors detecting the odour (Linssen, Janssens, Roozen, & Posthumus, 1993). Modified frequency (MF) is a method proposed by Dravnieks (1985) and is calculated as shown below.

$$MF(\%) = \sqrt{F(\%) \times I(\%)}$$

where F(%) is the detection frequency of an aromatic attribute expressed as percentage and I(%) is the average intensity expressed as the percentage of the maximum intensity. Van Ruth (2001) reported a review on different methods for GC-O. According to the author, the main drawbacks of the dilution techniques were the difficulty to use more than one assessor because of the lengthy process of the method and the invalidity of the two dilution factor assumptions. There is a nonlinear relationship between the perceived intensity of a compound and its concentration and the slopes for different odour-active compounds are different. According to Van Ruth and ÓConnor (2001), detection frequency and posterior intensity methods gave better correlations with sensory intensities-compund concentrations compared to dilution techniques.

Different approaches have been used for GC–MS data analysis. These include relative concentrations of compounds determined by using one internal standard (Cano-Salazar, López, & Echeverría, 2013; Lee, Vázquez-Araújo, Adhikari, Warmund, & Elmore, 2011; Xiao et al., 2014), relative peak areas from chromatograms (Andreu-Sevilla, Mena, Martí, Viguera, & Carbonell-Barrachina, 2013; Liu et al., 2015), and also concentrations determined by generating standard curves for each measured compound (Aznar, López, Cacho, & Ferreira, 2003; Green, Parr, Breitmeyer, Valentin, & Sherlock, 2011). In the case of using GC–MS and sensory data, better results could be achieved if the content of the aroma compounds is considered in relation to their odour thresholds, as this would express the importance of the individual aroma contributions (Varming et al., 2004).

2.2. Sensory analysis

Descriptive sensory analysis with selected sensory attributes is dominantly used in regression modelling. There are several descriptive sensory analysis methods available, such as QDA, Spectrum, Flavour Profile, etc. (Lawless & Heymann,, 2010). Research on whether or not one method provides better data for regression models over the others was not found in current literature. However, some descriptive sensory analysis methods that use untrained panellists or panellists with little training, such as Flash Profile or Free Choice Profiling may be more difficult to associate with instrumental data because of the high variability in the sensory data (Lawless & Heymann, 2010). Furthermore, sensory data from consumers may be collected to create regression models, as shown by Morita et al. (2015). The study used 59 housewives to evaluate Cheddar cheeses for palatability. The scores were

associated with GC-O data. One of the considerations when using consumer data is to collect data on attributes that are uniformly interpreted by all consumers, which may limit the usefulness of the sensory data.

When creating models to predict sensory data from analysis of volatiles, one should consider whether to include aroma or flavour results from sensory analysis. Aprea et al. (2012) found that the models built with flavour attributes were less stable and gave poor results. The authors explained that flavour is an interaction between volatile compounds, taste and texture; thus, they only presented the model with odour attributes. While this may be true, the decision of whether to use odour or flavour sensory data in the regression model depends on the objective of the study as well. For example, Koppel, Adhikari, and Di Donfrancesco (2013) used sensory smell data in finding associations among dry pet food volatile compounds and sensory characteristics. The objective was to determine which volatiles potentially cause which aromatic sensations. In another study Koppel, Gibson, Alavi, and Aldrich (2014) used sensory flavour data to find associations with volatile aromatics of baked and extruded pet foods. This approach can be justified by sample preparation methods. For GC analysis, the sample was ground and mixed with water; in flavour analysis, the dry sample was chewed in the mouth and mixed with saliva. Di Donfrancesco, Koppel, and Chambers (2012) showed that for pet foods, sensory flavour analysis provided more detailed data than sensory smell analysis alone. The approach that is selected depends on the researcher, the study at hand, and the analysis capabilities that are available.

3. Data pre-processing

3.1. Instrumental analysis

Data pre-processing is an essential part of chemometric data analysis. It can be separated into two main directions: removing data artifacts and transforming/rescaling the data by using a function. The choice of an optimal pre-processing method, or combination of methods, may strongly influence the results; but, it is not straightforward, since it depends on the characteristics of the data set and the goal of data analysis (Engel et al., 2013).

The most widely used method in data pre-processing is autoscaling (Bansleben et al., 2009; Mimura, Isogai, Iwashita, Bamba, & Fukusaki, 2014; Niu et al., 2011). This combines mean-centring and standardisation (dividing with standard deviation); thus, it gives equal weight to each variable. It is important to autoscale the data, especially in the cases where data are in different units and/or large deviations in the matrixes are present. Otherwise, more importance could unintentionally be given to the variables that have higher values or bigger fluctuations between the samples in terms of absolute values. Mean-centring and standardisation have also been used separately (Cheong, Liu, Zhou, Curran, & Yu, 2012; Vilanova, Genisheva, Masa, & Oliveira, 2010; Viljanen, Heiniö, Juvonen, Kössö, & Puupponen-Pimiä, 2014). Mean-centring is used to enhance the differences between samples and to remove the magnitude effects.

Pareto scaling is very similar to autoscaling. The difference is that instead of the standard deviation, the square root of the standard deviation is used. Therefore, large fold changes are decreased more than small fold changes. As a result, large fold changes are less dominant in clean data. After Pareto scaling, the data does not become dimensionless like after autoscaling (Van den Berg, Hoefsloot, Westerhuis, Smilde, & van der Werf, 2006).

Log-transformation has also been used to pre-process data (Aprea et al., 2012). Because the relationship between chemical concentration and sensory impact is nonlinear, Schulbach et al. (2004) suggested that when using GC–MS results, peak areas need to be additionally adjusted before analysis using log or square root transformations. Wold, Sjöström, and Eriksson (2001) claimed that variables with a range of more than a magnitude of 10 are often logarithmically transformed to make distribution fairly symmetrical. There are few studies comparing the results of different pre-processing techniques. For example, da Silva et al. (2012) performed partial least squares regression (PLSR) on data with (a) no pre-processing, (b) mean-centred and (c) autoscaled data, and concluded that the best performance was obtained with autoscaled data. Chung, Heymann, and Grün (2003) found that for PLS, log-transforming the data set was not able to sufficiently predict the effect of flavour compounds on the sensory attributes of ice cream flavour. Better performance was gained with the original data of chromatographic peak areas. When using generalised Procrustes analysis (GPA), log-transformation can be helpful in correlating chemical and sensory relations.

Typical data artifacts related to chromatography are baseline offset and slope, misalignment and noise. Different algorithms can be used to improve the data. For example, Ribeiro, Augusto, Salva, Thomaziello, and Ferreira (2009) used the Savitzky-Golay algorithm (Savitzky & Golay, 1964) for noise reduction and correlation optimised warping (COW) to handle peak shifts. The COW method splits the signal into different segments and optimal alignment should be reached by stretching or compressing the individual segments to better match the reference segments (Engel et al., 2013). For further reading on pre-processing techniques, see the review by Pierce, Kehimkar, Marney, Hoggard, and Synovec (2012).

Unfortunately, most published studies do not point out whether any pre-processing techniques have been applied to the original GC/MS chromatograms. However, the exact information on the treatment of the raw data submitted to statistical analysis is essential and should be included in the experimental section of scientific papers in the future.

3.2. Sensory analysis

Data preprocessing or preselection may occur in descriptive sensory analysis as well. However, in most publications, this would not be mentioned or specified. For example, the data may be screened for panellists who are outliers, meaning they scored the samples in a considerably different manner from the bulk of the panellists. This may result in partial or total exclusion of those scores from the dataset. For example, da Silva et al. (2012) checked data sets by analysis of variance (ANOVA) and Student's *t*-test to find possible inconsistencies and outliers. Sensory scientists often try to avoid excluding any panellists from the final dataset. This is achieved by training the panellists, orienting the panellists to the samples to be tested, and pre-testing some of the samples. Sometimes the data are screened for samples that are scored considerably different from the rest of the samples for some attributes. This is often done using multivariate methods such as principal components analysis (PCA). Depending on the sample map created from the PCA, this may result in some of the samples being excluded from the final dataset. Sensory data may be checked for mistakes, i.e. scores that are obviously entered as a random mistake, and missed values, i.e. scores that were missed during evaluation. The handling of these type of mistakes is dependent on the researcher and the software used for data analysis. But again, in most publications these types of data pretreatments would not be mentioned.

4. Variable selection

Variable selection is used to get better correlations between explanatory and independent variables and to improve the performance and prediction capability of the model. With many

variables being irrelevant, noisy or unreliable, removal of these will typically improve the predictions and/or reduce the model complexity (Andersen & Bro, 2010).

4.1. Instrumental analysis

Variable selection could be based on statistical techniques or, more subjectively, based on the prior knowledge of the variables. For example, GC/O data from Liu et al. (2015) used the flavour dilution factor according to AEDA analysis procedure to determine predominant odour-active compounds. Another possibility is to set a value of odour detection frequency (Bansleben et al., 2009) for which compounds below the threshold are excluded. The same approach has been used for the sum of posterior intensities (Thomsen et al., 2012) and the modified frequency method (Campo et al., 2005).

When working with GC–MS data, many authors have included the correlation analysis compounds that have an odour activity value (OAV = concentration/odour threshold) above a specified number (Aznar et al., 2003; Vilanova et al., 2010, 2012; Xiao et al., 2014). For example, Vilanova et al. (2012) only included compounds that have an OAV greater than 0.2. It may be assumed that only the compounds that are present in concentrations above the odour threshold are important in terms of sensory perception. In practice, it is necessary to take into account the accuracy of the odour threshold value, the determined concentration and the effect of the matrix of the sample. Also, the synergy of similar odour-active compounds, which could have an impact on flavour when occurring together, should not be underestimated. Therefore, including compounds with rather low OAVs in the statistical analysis could be reasonable. The selection of GC–MS peaks to include in the statistical analysis could also be based on GC-O results. For example, Owusu et al. (2013) used GC-O data for selecting 16 of the most important odour-active compounds from GC–MS data. The latter were determined by selecting compounds detected by all eight judges in at least one sample (detection frequency method).

The most widely used statistical technique to evaluate the contribution of each variable and to test significant differences of variables among samples is analysis of variance (ANOVA). Generally, ANOVA is applied to sensory data, but it has also been used to get an overview of which instrumental variables have statistically significant differences among samples (Lignou, Parker, Baxter, & Mottram, 2014). Another method used to select the variables to include in the analysis and therefore improve the model performance is variable importance for the projection (VIP) value (Mimura et al., 2014; Ochi, Bamba, Naito, Iwatsuki, & Fukusaki, 2012). VIP values estimate the importance of each variable in the projection used in a PLS model. Most commonly the variables with a VIP value smaller than 1 are excluded from further analysis. When using VIP values, it should be kept in mind that when running an analysis there will always be variables with VIP value smaller than 1. This is due to the fact that the average of VIP values for all variables is always 1. There are also other statistical approaches to determine statistically important components, such as genetic algorithms (GA) and ordered predictors selection (OPS), but these are not widely used in the field of correlating sensory and volatiles data. da Silva et al. (2012) compared the variables selected by using both GA and OPS to predict two sensory attributes. Those authors found that 64–67% of the variables selected with GA were also selected by using OPS. The selection of variables should be performed carefully as there is a high risk of overfitting the data. If totally new data is introduced to the model, it could fail to predict the results. For further reading concerning statistical methods for variable selection, see Andersen and Bro (2010) and Mehmood, Liland, Snipen, and Sæbø (2012).

4.2. Sensory analysis

In some studies, assessors have generated descriptive terms during the sensory session. In that case, geometric mean (GM) could be used to evaluate which sensory attributes are the most important in terms of describing the samples and therefore, to include in further analysis (Liu et al., 2015; Vilanova et al., 2010, 2012). GM takes into account how many assessors have recognised the attribute and also how they have rated the intensity of the attribute. For several descriptive sensory analysis methods, all of the panellists should be trained and understand the attributes in the same way. For example, the Flavour Profile and the Spectrum methods require extensive training and orientation of the panellists before evaluations are conducted. This should preclude the need for GM calculation and the arithmetic mean can be used instead. As long as good practices are used in attribute creation, as described by Lawless and Heymann (2010), all attributes should be important in the final dataset.

In case of sensory data, ANOVA has been used to get an overview of which variables have statistically significant differences among samples (Niu et al., 2011), but also to exclude the variables with no statistical difference among samples (Liu et al., 2015; Mimura et al., 2014).

5. Statistical methods for correlating sensory and volatiles data

Partial least squares regression (PLSR) is the most widely used statistical tool in correlating sensory and instrumental data and creating prediction models (Table 1). A few authors have used correlation coefficients to assess the relations (Vilanova et al., 2010; Xiao et al., 2014) or compared clustering of volatiles and sensory attributes by hierarchical cluster analysis (HCA) (Aprea et al., 2012). These two methods can show possible associations but could not be used to predict sensory qualities of new samples by using gas chromatographic data of volatiles.

5.1. Partial least squares regression

PLSR is a method for relating two data matrices, *X* and *Y*, and uses latent variables to model the covariance of matrixes *X* and *Y*. PLSR can analyse data with noise, colinearity and missing variables in both *X* and *Y* matrices. It also does not require the number of samples to be higher than the number of variables. When increasing the number of relevant variables with the PLSR method, the precision of the model parameters improves (Wold et al., 2001).

Before conducting PLSR, a decision must be made whether to make a model for each *Y*-variable separately (PLS1) or to make one model including all the *Y*-variables (PLS2). Hence, according to Wold et al. (2001), one should start with a PCA of just the *Y*-matrix. If the number of resulting principal components is small compared to the number of original *Y*-variables, it means the *Y*-variables are correlated and a single PLS model for all *Y*-variables is warranted. If *Y*-variables are clustered in groups, different models for each group should be developed. If the variables are scattered all over the PCA plot, this means that it would be reasonable to perform analysis for each *Y*-variable separately.

The number of components included in PLSR analysis is usually determined by cross-validation (CV). CV is performed by dividing the data in a number of groups and then developing a number of parallel models from reduced data with one of the groups deleted (Wold et al., 2001). The most often used CV is called leave-one-out CV (LOOCV). In the case of LOOCV, each sample is left out once and used for validation. LOOCV has been characterised with rather high variance and low bias compared to other types of CV procedures

Table 1
Examples of multivariate analysis on relations of sensory and gas-chromatographic data carried out since 2009.

	Sample	Number of samples	Extraction method	Instrumental technique	Type of instrumental data	Data preprocessing	Statistical method for correlation	Results	Model quality	References
1	Oregano	3	Stir-bar-sorptive extraction (SBSE)	GC-O-FID	Detection frequency FID peak area	Autoscaling	PLSR, FA	For predicting the right class of specimen with PLSR for specimen 2, errors are 11% and 4%	No data	Bansleben et al. (2009)
2	Cherry wine	9	HS-SPME	GC-TOF-MS	Relative concentrations	No	PCA, correlation analysis	First 2 principal components explained 69.99% variance of volatiles and 97.61% variance of sensory attributes. Reasonable conclusions could be drawn based on received correlation coefficients	No data	Xiao et al., 2014
3	Australian Cabernet Sauvignon	30	HS-SPME	GC × GC–MS	Relative concentrations	Normalising (each product both *x* and *y* variables) against maximum value	PLSR (PLS1), two-way HCA	4 PLS components explained 97–99% of *y*-variance. *x*-variance not well explained (from 8–77%). 303 volatiles clustered in 30 groups based on correlation coefficients	No data	Robinson et al. (2011)
4	Sake	40	SBSE/SE	GC–MS	Relative concentrations	Autoscaling	OPLSR (PLS 1)	Explained variance of models were higher than 90%	r_{cv} = 0.905–0.963	Mimura et al. (2014)
5	Arabica Coffee	58	HS-SPME	GC–MS-FID	Chromatograms	Alignments (COW method), smoothing (Savitzky–Golay algorithm, autoscaling, mean-centred	PLSR	When using the number of latent variables determined from RMSECV values for all models, variance explained was in general 95% in case of *y* and 52% for *x*-matrix	RMSECV 0.18–0.39. r_{cv} = 0.88–0.91	Ribeiro et al. (2009)
6	Pomelo juice	2	HS-SPME/SE	GC–MS-FID	Relative concentrations	Standardisation	PLSR	The first 2 PLSR components explained 21–86% of *y*-variance	No data	Cheong et al. (2012)
8	Sauvignon blanc wine	18	HS-SPME	GC–MS	Concentrations	No information	PLSR	The first 2 PLSR components explained 67% of *x*-variance and 65% of *y*-variance. Perceptual separation between different origins	No data	Green et al. (2011)
9	Fruit smoothie	12	HS-SPME	GC–MS	Relative concentrations	No information	PLSR	Poor correlation, coefficients $r < \pm 0.3$	No data	Keenan, Brunton, Mitchell, Gormley, and Butler (2012)
10	Cherry wine	5	DCM extraction	GC-O/MS	Relative concentrations	Autoscaling	Anova PLSR	The first 2 PLSR components explained 63% of *x*-variance and 59% of *y*-variance. Mostly Pearson correlation coefficients > ±0.5	No data	Niu et al. (2011)
11	Semi-hard cheese	7	Purge and trap	8W-GC-O	Sum of GC-O intensities	Mean-centring	PLSR	The first 2 PLSR components explained 68% of x-variance and 89% of *y*-variance	No data	Thomsen et al. (2012)
12	*Makgeolly* (rice wine)	12	HS-SPME	GC–MS	Relative concentrations	No information	PCA	8 volatiles out of 45 correlated with sensory attributes well. Pearson correlation coefficients > ±0.7	No data	Jung, Lee, Lim, Kim, and Park (2014)
13	*Godello* (white wine)	5	SPE	GC–MS	Relative concentrations	No information	PLSR (PLS1 and PLS2)	The first 2 PLSR components explained 98% of *x*-variance and 51% of *y*-variance. PLS1: *y*-variance was explained 44–79%	RMSEP lower than 10 for all models	González-Álvarez, González-Barreiro, Cancho-Grande, and Simal-Gándara (2011)

14	Bioprocessed lingonberry	6	HS-SPME	GC–MS	Relative concentrations	Standardisation, normalisation	PLSR	The first 2 PLSR components explained 88% of *x*-variance and 90% of *y*-variance	No data	Viljanen et al. (2014)
15	Hard and semi-hard cheese	13	SE	GC–MS, GC-FID	Relative concentrations	Pareto scaling, auto-scaling, centring	PLSR (PLS1)	Models for 2 chosen sensory attributes explained both more than 95% of *y*-variance	RMSEP 0.136–1.248 and r_{cv} = 0.919–0.985	Ochi et al. (2012)
16	Solaris white wines	12	DHS	GC–MS, GC-FID	Semi-quantified (relative peak areas)	Autoscaling	PLSR	The first 2 PLSR components explained 44% of *x*-variance and 78% of *y*-variance	No data	Liu et al. (2015)
17	Red wine	57	SE/SPE	GC-FID	Concentrations	No information	PLSR (PLS 1)	Satisfactory models (explained variance >45%) gained half of the sensory attributes	RMSEP = 0.81–2.71 and r_{cv} = 0.2–0.81	Aznar et al. (2003)
19	Walnuts	4	HS-SPME	GC–MS	Relative concentrations	No information	PLSR	The first 2 PLSR components explained 45% of x-variance and 51% of *y*-variance	No data	Lee et al. (2011)
20	*Vitis vinifera* red cultivars	5	SE/SPE	GC-FID, GC-ion trap-MS	Relative concentrations	Standardisation	PLSR (PLS 1)	In case of 4 important sensory parameters correlated to instrumental data, the explained variance of models were higher than 99.6%	No data	Vilanova et al. (2012)
21	Pomegranate juice and wine	6	HS-SPME	GC–MS	Semi-quantification (relative peak areas)	No information	PLSR	The first 2 PLSR components explained 86% of x-variance and 80% of *y*-variance	No data	Andreu-Sevilla et al. (2013)
22	Apple	18	HS-SPME	GC–MS	Relative concentrations	Log-transformation, mean-centering, normalisation	PLSR (PLS 1), HCA	Apples clustered in very similar groups based on sensory results and volatiles. *y*-variance explained was 0.52–0.95%	R_{cv} = 0.52–0.95	Aprea et al. (2012)
23	Peach, nectarine	4	Dynamic HS	GC-FID	Concentrations	Autoscaling	PLSR	Correlated in the same models besides volatiles also different physiochemical properties	No data	Cano-Salazar, López, and Echeverría (2013)
24	Chocolate	6	Dynamic HS	GC–MS GC-O-FID	Semi-quantification	Autoscaling	PLSR (PLS1 and PLS2)	PLS2 components 1 and 2 explained 86% of total variance. PLS1 best results got attribute fruitiness (80% explained variance)	RMSECV = 1.62 and r_{cv} = 0.55	Owusu et al. (2013)
25	Spanish Albarino wines	35	SE	GC-FID	Relative concentrations	No information	Pearson correlation coefficient, PLSR	Only 5 Pearson correlation coefficients between sensory attributes and volatiles were larger than 0.5. PLS components 1 and 2 explained 45% of total variance	RMSEP = 9–14	Vilanova et al. (2010)
26	Chocolate	9	SAFE	GC/O/MS	Concentrations	No information	PLSR	The first 2 PLSR components explained 97.8% of *x*-variance and 88.3% of *y*-variance	No data	Liu et al. (2015)
27	Beer	32	HS-SPME	GC/MS	Peak areas	Autoscaling	PLSR (PLS 1)	R^2 for two analysed sensory parameters were between 0.8851–0.9678	RMSECV = 0.25–0.33	da Silva et al. (2012)
28	Espresso	18	Dynamic HS	GC/O	Charm values	No information	ANN, PLSR	Analysing 10 odour and 4 sensory descriptors with PLS R^2 was 0.55 and ANN R^2 was 0.79	With PLSR r_{cv} = 0.35 and with ANN r_{cv} = 0.77	Michishita et al. (2010)

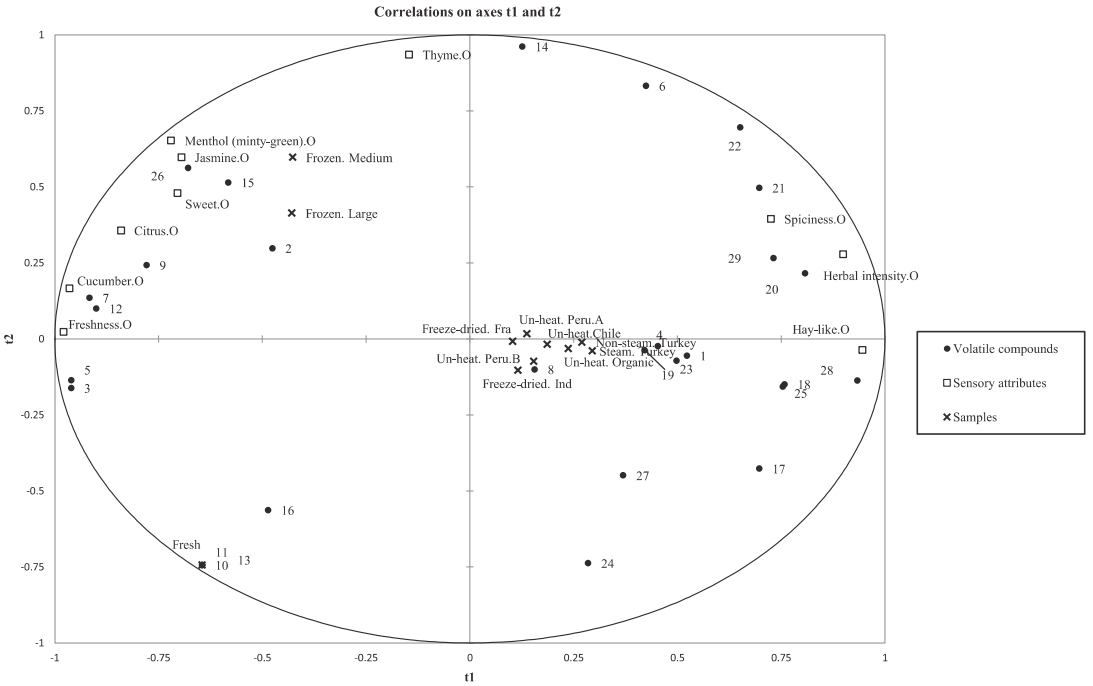

Fig. 1. PLSR biplot of oregano samples from different origins and different drying methods applied.

with lower number of validation datasets (Arlot & Celisse, 2010; Shao, 1993). Therefore, the selection of the most suitable CV procedure is highly dependent on the characteristics of the data and the purpose of analysis.

Fig. 1 is an example of a typical PLSR biplot, where explanatory variables (volatile compounds numbered 1–29), dependent variables (sensory data) and analysed samples (oreganos varying in origin and dried by using different methods) are mapped together. The instrumental data used in the analysis are the sum of the intensities of GC/O results which have been autoscaled prior to PLSR analysis. Sensory data are based on panel averages from descriptive analysis without excluding any information. Volatile compounds with VIP score below 0.8 were excluded from the final analysis, as well as sensory odour attributes with cumulative Q^2 below 0.4. The number of components to include in the analysis was determined by using cross-validation. As seen in Table 2, Q^2 is the highest with five components and decreases with adding a sixth component. *X*-variance and *Y*-variance explained by the model is increasing with each component but the decrease in Q^2 value with the sixth component indicating that overfitting has occurred. The first two components of the model explained 64% of *X*-variance and 83% of *Y*-variance. Different associations could be seen from the biplot (Fig. 1). Firstly, grouping of the samples: fresh and frozen samples are differentiated from the other heat and non-heat treated dried samples. Secondly, correlations between sensory attributes and volatiles can be found. For example, volatile compounds 7 and 12 have a strong positive correlation with sensory attributes "freshness" and "cucumber". Thirdly, correlations between samples and sensory attributes, and samples and volatile compound can be observed. For example, fresh samples have the best correlation with volatiles 10, 11, 13 and 16.

Table 2
PLSR model quality.

Index	Comp1	Comp2	Comp3	Comp4	Comp5	Comp6
Q^2 cum	0.426	0.728	0.756	0.762	0.797	0.783
R2Y cum	0.634	0.862	0.918	0.941	0.966	0.978
R2X cum	0.433	0.649	0.735	0.845	0.892	0.920

5.2. Artificial neural networks (ANN)

While PLSR is widely used for visualising correlations between datasets, ANN is a method which is used only for composing prediction models. ANN is a method that tries to simulate the way a human brain works. The model consists of different layers of neurons – input layer, hidden layer(s) and output layer. There are connections between neurons in each layer. When dealing with sensory–instrumental relations, the input layer could be considered as independent variables (volatile compounds) and the output layer as dependent variables (sensory attributes). The backpropagation algorithm (Rumelhart, Hinton, & Williams, 1986) is widely used in layered feed-forward ANNs. This means that the artificial neurons, which are organised in layers, send their signals "forward" and then the errors are propagated backwards. The input to the network is received by neurons in the input layer, and the output of the network is given by the neurons in an output layer. In between there are one or more hidden layers. The backpropagation algorithm uses supervised learning, which means that we provide the algorithm with examples of the inputs and outputs we want the network to compute, and then the error is calculated. The training begins with random weights. The goal is to adjust them so that the error will be minimal (Šibalija & Majstorović, 2016). Therefore, by using a training dataset, we can create a model

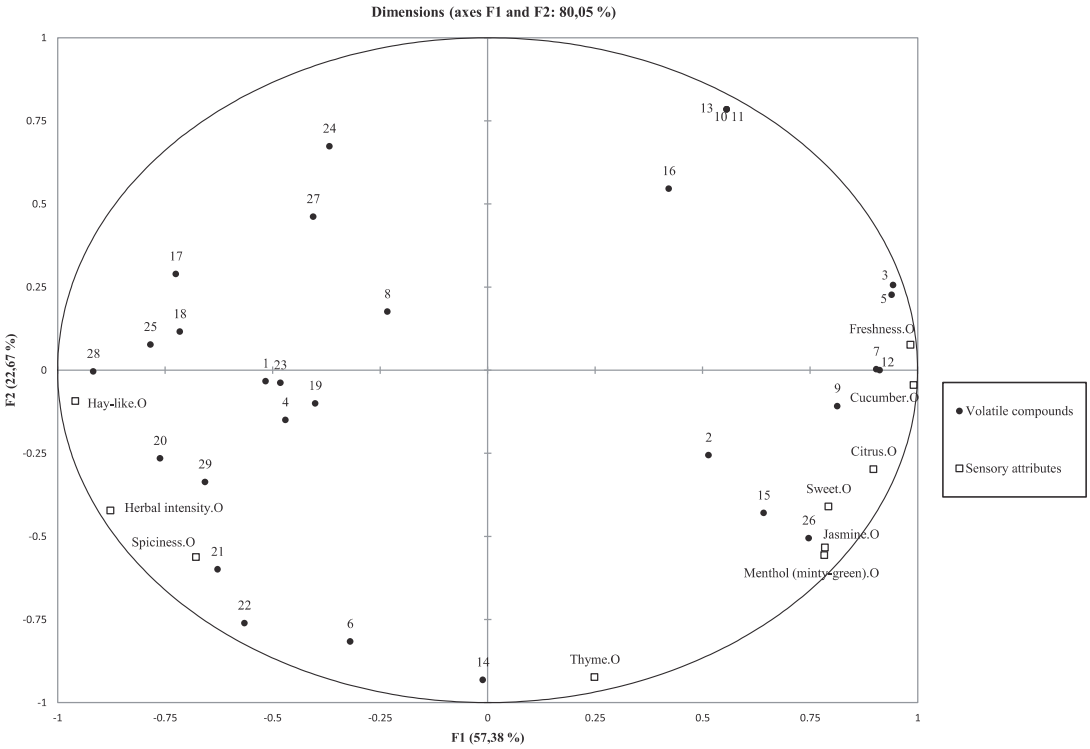

Fig. 2. GPA biplot of sensory attributes and volatile components of oregano samples from different origins and different drying methods applied.

which has a sufficient number of hidden layers transformed by different mathematical algorithms to predict sensory characteristics from volatiles data. After this, the reliability of the model with new known data can be tested and used for predicting sensory parameters of unknown samples. The main advantage of ANN over PLSR is the capability to model non-linear relations. Many authors predicted in the 1990s that ANN would gain a wider field of application in the area of correlating sensory–instrumental relations (Macfie & Hedderley, 1993; Peppard, 1994). However, in the last 20 years there have been only a few studies using neural networks for modelling sensory–instrumental relations (Boccorh & Paterson, 2002; Cancilla et al., 2014; Michishita et al., 2010). One reason for this is that the number of samples must be higher than the number of variables in ANN. Theoretically, the number of samples should be four times higher than the number of variables (Peppard, 1994). Because sensory analyses are often time consuming, the sensory data gathered is quite small. Thus, the selection of statistical methods should be planned out together with the study objectives, samples, and analysis methods.

5.3. Generalised Procrustes analysis (GPA)

GPA is a method, which can be used to find a common structure between two datasets like sensory and volatiles data. It has been used in many earlier studies (Chung et al., 2003; Le Fur, Mercurio, Moio, Blanquet, & Meunier, 2003). It can be a useful method for investigating relations between sensory and volatiles data, but it cannot be used to create predictive models. The objective of GPA is to try to get the same objects as close to each other as possible by shifting entire configurations, as well as rotating and reflecting them if necessary. When the configurations are stretched or shrunk, the relative distances between the objects remain the same (Dijksterhuis, 1996). In terms of correlating sensory and instrumental data, the datasets of sensory results and volatile compounds can be seen as different configurations, which need to be converged. To illustrate the possibilities of GPA, the authors have applied GPA to the same dataset of different oregano samples that was used for PLSR previously. Also, the same preprocessing was applied. A correlation biplot very similar to the one with PLSR was obtained (Fig. 2). The first two components explained 80% of total variance. From the biplot, correlations between sensory attributes and volatile compounds could be observed. Fig. 3 represents the map of samples, where each sample is mapped with three markers: based on sensory results, based on GC-O results and the consensus spot of sensory and GC-O results. This map gives an indication of how the samples were grouped and also shows the correlations between patterns in sensory data and GC-O results. Fig. 4 represents the residues of the samples after transformations. For example, samples *Frozen.medium* and *Freeze-dried.Ind.* had the highest residuals, which means that the correlation between sensory and GC-O data for these two samples were the smallest.

6. Model validation

Data analysis is often based on evaluation of fitted models. Validation can be seen as the part of the analysis where it is investigated if valid conclusions can be drawn from a model (Smilde, Bro, & Geladi, 2004). The first indications of the model quality

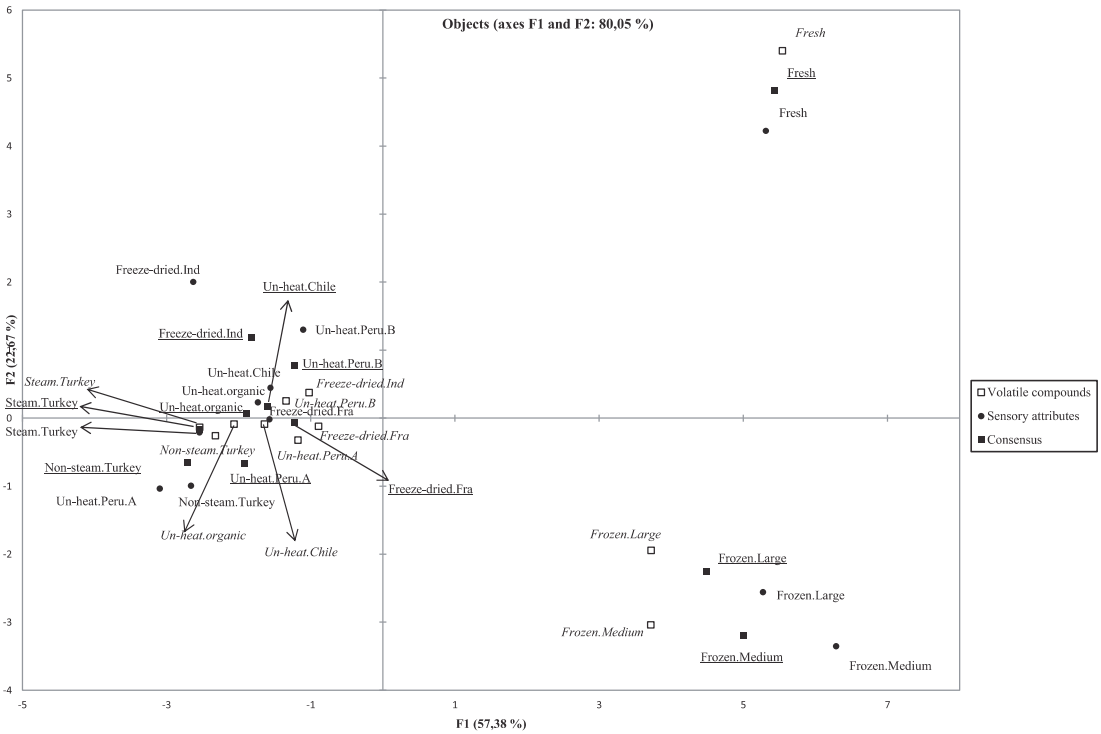

Fig. 3. GPA biplot of oregano samples from different origins and different drying methods applied based on sensory data, volatile compounds and consensus coordinates.

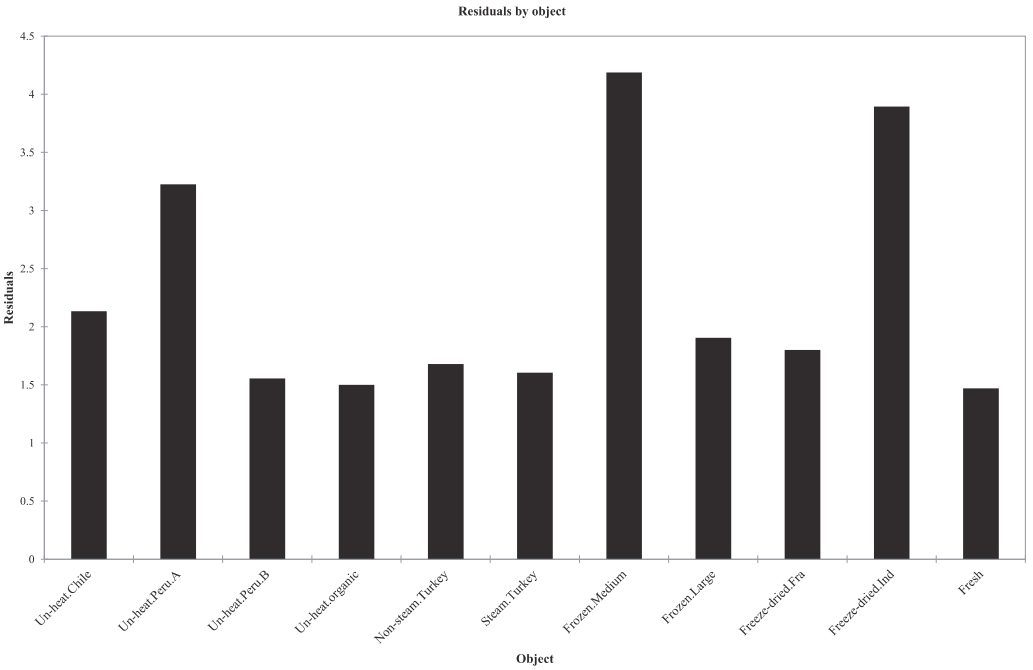

Fig. 4. GPA residuals for samples of oreganos from different origins and different drying methods applied.

are the values of R^2 and Q^2. R^2 displays the fraction of the sum of squares for the selected component. It also shows the variance explained by the model. Q^2 shows the fraction of the total variation of Y that can be predicted by a component, as estimated by cross-validation. To see the calculation steps for R^2 and Q^2, see the study by Wold et al. (2001). In order to have a good model, it should have a good predictive and explanatory power (high R^2 and Q^2 values). There are several possibilities to assess the predictive capability of models. One of the possibilities is to use a validation dataset. This can be done with the data that was preliminary excluded from the original dataset when computing the models or, even better, with totally new dataset. The amount of data for training and testing can be limited. In order to build good models, it is better to use as much of the available data as possible for training. If the validation set is small, it will give a relatively noisy estimate of predictive performance. There are multiple data resampling techniques to solve these problems, from which cross-validation is one very widely used solution (Aprea et al., 2012; Aznar et al., 2003; Mimura et al., 2014).

When using cross-validation, the same data are used for composing the model and also for the validation. The model is validated by leaving part of the data out when creating the models, and the data left out are later used to test the model. The same procedure is repeated until all of the data have been left out once. CV is a good choice to get an indication of how reliable the correlations between sensory and instrumental data are and to get the first indication of model performance. Still, to build a robust prediction model, validation with new data is necessary in order to get true predictions. Model performance is most commonly characterised by using root mean square error (RMSE). If cross-validation is used, it is marked as root mean square error of cross-validation (RMSECV); if external validation dataset is used, it is marked as root mean square error of prediction (RMSEP). For instructions on the calculation of RSMSECV and RMSEP, see Ribeiro et al. (2009).

7. Future perspective

As the association between volatiles and sensory perception is often nonlinear, in future studies different nonlinear techniques should be tested. As already mentioned, ANN could be a good perspective, but due to its limitation of needing large datasets, it is unlikely to gain popularity in investigating sensory–instrumental relations. One promising alternative could be the application of the nonlinear extensions of PLSR, like Kernel PLSR (Rosipal & Trejo, 2001) or second order polynomials (Wold, Kettaneh-Wold, & Skagerberg, 1989). Kernel methods have been used extensively in machine learning applications (Hofmann, Scholkopf, & Smola, 2008). It is also rather popular in chemometrics, but so far not used in correlating sensory–instrumental relations. In kernel PLS, data from the X-matrix are nonlinearly mapped into new high-dimensional space, where linear PLS is then implemented.

8. Conclusions

Although statistical methods are widely used to correlate sensory and gas chromatographic data, information on the treatments applied to the original data set and also the validation results are often inadequate or missing. There is limited information on different variable selection techniques used and very little research conducted on comparing the results gained with different techniques. VIP or ANOVA appeared as the most used methods. Moreover, PLSR is shown as the most common method for correlating and calculating the models of sensory and gas chromatographic data. The main advantages of PLSR are the ability to measure the covariance of two matrices and to analyse the data with multicolinearity and missing values. The main disadvantage of PLSR is that it only models linear relations. In many publications even when the models or correlation parameters are described, the indicators of the reliability of the results like the values of Q^2 and RMSE have not been mentioned. As the relation between volatiles concentration and sensory perception is not linear, future studies could look at non-linear methods for analysing the relations between sensory and volatiles data.

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PUBLICATION IV

Kortesniemi, M., **Rosenvald, S**., Laaksonen, O., Vanag, A., Ollikka, T., Vene,K., Yang, B.

Sensory and chemical profiles of Finnish honeys of different botanical origins and consumer preferences.

Food Chemistry, in press, accepted manuscript.
https://doi.org/10.1016/j.foodchem.2017.10.069

Accepted Manuscript

Sensory and chemical profiles of Finnish honeys of different botanical origins and consumer preferences

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PII: S0308-8146(17)31707-7
DOI: https://doi.org/10.1016/j.foodchem.2017.10.069
Reference: FOCH 21892

To appear in: *Food Chemistry*

Received Date: 6 July 2017
Revised Date: 10 October 2017
Accepted Date: 10 October 2017

Please cite this article as: Kortesniemi, M., Rosenvald, S., Laaksonen, O., Vanag, A., Ollikka, T., Vene, K., Yang, B., Sensory and chemical profiles of Finnish honeys of different botanical origins and consumer preferences, *Food Chemistry* (2017), doi: https://doi.org/10.1016/j.foodchem.2017.10.069

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ACCEPTED MANUSCRIPT

Sensory and chemical profiles of Finnish honeys of different botanical origins and consumer preferences

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Abstract

The sensory-chemical profiles of Finnish honeys (labeled as buckwheat, cloudberry-bog, lingonberry, sweet clover, willowherb and multifloral honeys) were investigated using a multi-analytical approach. The sensory test (untrained panel, $n = 62$) was based on scaling and check-all-that-apply (CATA) methods accompanied with questions on preference and usage of honey. The results were correlated with corresponding profiles of odor-active compounds, determined using gas chromatography coupled with mass spectrometry/olfactometry (GC–MS/O). Botanical origins and chemical compositions including sugars were evaluated using NMR spectroscopy. A total of 73 odor-active compounds were listed based on GC–O. Sweet and mild honeys with familiar sensory properties were preferred by the panelists (PCA, $R^2X(1) = 0.7$) while buckwheat and cloudberry-bog honeys with strong odor, flavor and color were regarded as unfamiliar and unpleasant. The data will give the honey industry novel information on honey properties in relation to the botanical origin, and consumer preference.

Keywords

Botanical origin; CATA; Chemical profile; Consumer preference; Honey; Odor-active compound; Sensory profile

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1. Introduction

Botanical origin has a great impact on the sensory, physicochemical and bioactive properties of floral honeys. The origin of the crude material, the nectar, has a major contribution on the color, flavor, odor and texture of the honey (Piana, Persano Oddo, Bentabol, Bruneau, Bogdanov & Guyot Declerck, 2004; da Silva, Gauche, Gonzana, Costa, & Fett, 2016). The boreal biotope in Finland is the source of unique aromas, but also the reason for the challenges encountered in the production of pure varietal honeys. The origin of honey is crucial when assessing its quality, authenticity, bioactive potential and commercial value. Since most honeys are only characterized by the beekeeper's personal evaluation, the sensory characteristics of different honey types should be well known. However, the natural variability of honeys may complicate their characterization (Piana et al., 2004). In honeys of mixed botanical origins, the strong sensory characteristics of one botanical source can dominate the mild properties of another source even at low proportions and change the overall sensory profile of the honey (Piana et al., 2004).

Blossom and more specifically varietal honey is defined in the Council Directive 2001/110/EC and its amendment 32014L0063 from 2014 (European Union, 2002 & 2014). Varietal honeys are generally identified based on sensory (Piana et al., 2004; González Lorente, De Lorenzo Carretero, & Pérez Martín, 2008; Stolzenbach, Byrne, & Bredie, 2011; Silvano, Varela, Palacio, Ruffinengo, & Yamul, 2014), melissopalynological (Louveaux, Maurizio, & Vorwohl, 1978; von der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004) and various physical and chemical analyses (Persano Oddo, Piazza, Sabatini, & Accorti, 1995; Anklam, 1998; da Silva et al., 2016). Identifications can be based on composition and contents of certain compound groups, such as amino acids (Rebane, & Herodes, 2008) or phenolic compounds (Ciulu, Spano, Pilo, & Sanna, 2016; Zhao et al., 2016), or on the metabolome of the honey using, for example, NMR analyses (Spiteri et al., 2015; Kortesniemi et al., 2016; Schievano, Finotello, Uddin, Mammi, & Piana, 2016; Spiteri et al., 2017). Plant-derived compounds contributing to the odor and flavor of the honey can also serve as

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indicators of the botanical origin (De la Fuente, Sanz, Martínez-Castro, Sanz, & Ruiz-Matute, 2007; Castro-Vázquez, Díaz-Maroto, González-Viñas, & Pérez-Coello, 2009; Seisonen, Kivima, & Vene, 2015). Harmonized methods and terminology in sensory analysis of honeys are established for a selection of European honeys (Piana et al., 2004; International Honey Commission, 2009). The sensory and chemical characteristics of Finnish raspberry, willowherb, lingonberry and cloudberry-bog (mire) honeys have been investigated earlier by Salonen et al. (Salonen, 2011; Salonen, Hiltunen, & Julkunen-Tiitto, 2011; Salonen & Julkunen-Tiitto, 2012). Recently, studies focusing on volatile/aroma/odor-active compounds in honey or combining sensory and chemical analyses have been employed; for example, aroma extract dilution analysis (AEDA) and solid-phase microextraction (SPME) with GC–MS- and GC–O-based analytical methods (Ruisinger & Schieberle, 2012; Seisonen et al., 2015; Siegmund, Urdl, Jurek, & Leitner, 2017). These studies have covered, e.g., European alder buckthorn, chestnut tree, dandelion, fir tree, heather, lavender, linden tree, orange, rape and robinia honeys. Still, the compounds contributing to the odor and flavor of Finnish honeys have remained understudied until now.

The aim of this study was to determine the key flavor and odor descriptors and the corresponding odor-active compounds of selected honeys of different botanical origins from Finland. The purpose was also to afford useful information for beekeepers and the honey industry, by creating sensory-chemical honey profiles and providing consumer preference data. To our knowledge, this is the first sensomics-based study on Finnish honeys, combining gas chromatographic-mass spectrometric/olfactometric (GC–MS/O) methods and NMR spectroscopy/metabolomics with sensory analysis and consumer studies.

2. Materials and methods

2.1. Samples

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Seven honey samples from the 2014 harvest were acquired directly from beekeepers with the help of the Finnish Beekeepers' Association. The major floral sources of the honey samples were buckwheat (*Fagopyrum esculentum*) from Kitee (62° N), cloudberry-bog (biotope honey including *Rubus chamaemorus*) from Sodankylä (67° N), willowherb (*Epilobium* spp.) from Pihtipudas (63° N), lingonberry (*Vaccinium vitis-idaea*) from Muhos (64° N) and sweet clover (*Melilotus albus*) from Salo (60° N). Two multifloral honeys, smooth (of mainly Brassicaceae, *Trifolium* spp. and *Rubus* spp.) and granular (of mainly *Rubus* spp., *Trifolium* spp. and *Vaccinium* spp.), were also acquired for reference (Supplementary material **Fig. S1**). The botanical origins of the honeys were initially determined by the respective beekeepers, by assessing the characteristics of the honey (flavor, smell, color, texture) and the hive surroundings (hive location, dominant biotope, flowering). The botanical origins were verified with melissopalynogical analysis of 400 pollen grains per sample (Louveaux, Maurizio & Vorwohl, 1978; Salonen, Ollikka, Grönlund, Ruottinen & Julkunen-Tiitto, 2009) and by ^{1}H NMR spectroscopy (Kortesniemi et al., 2016). The samples were stored in a refrigerator (+4 °C), taken to room temperature over 24 h and mixed thoroughly prior to analyses.

2.2. Chemicals

Pure standards (GC grade) of dimethyl sulfide, (*E*)-β-damascenone, vanillin, methional were purchased from Merck (Schuchardt OHG, Germany). 1-Octen-3-ol, 2,3-butanedione, butanoic acid, phenylacetaldehyde, ethyl phenylacetate, hexane, heptane, 2-phenylethylacohol, (*E*,*Z*)-2,6-nonadienal were from Sigma-Aldrich. Kovats retention indices were determined using C8–C22 mix from Sigma-Aldrich. Internal standard solution containing DDS-d_6 (5 mM) and NaN_3 in D_2O was from Chenomx Inc. (Edmonton, Alberta, Canada) was used for preparing the samples for NMR.

2.3. Sensory evaluation

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The panel consisted of 62 panelists (35 females, 27 males) of age 15–71 years (mean 36.8 ± 13.2). The untrained panelists were given seven blind-coded, randomized honey samples, approx. 15 mL each, in lid-covered 100-mL glass vials to capture the headspace (Supplementary material **Fig. S1**). Each panelist was provided with a questionnaire of 30 questions related to demographic and usage (usage frequency, preferred honey type, honey origin, consumption type) information and the sensory characteristics of the samples, including attributes rated on scales, check-all-that-apply (CATA; Varela & Ares, 2012) questions and the preference of different honeys. The intensity of the odor, flavor, aftertaste and color together with sweetness, acidity and the familiarity of the odor and flavor were evaluated on a 5-point scale from 1 (not at all) to 5 (very strong). The main categories (12; each presented at a time on the computer program) of odor and flavor descriptors were "berry-like", "fruity", "floral", "herbaceous", "woody", "nutty", "spicy", "caramel", "earthy", "microbiological", "chemical" and "animal-like", featuring 147 descriptors in total. The sub-attributes for each category are presented in the supplementary **Table S1**. Each category included also the options "I don't know", "no attribute" and "attribute in general". The panelists had an opportunity to choose the most and the least liked samples and deliver additional descriptors and comments on the samples. The data were collected using Compusense® five version 5.2 data collection software (Compusense Inc., Guelph, ON, Canada). Tests were conducted in controlled sensory laboratory conditions in accordance with ISO8589:2007 standard.

2.4. Solid-phase microextraction

A dilution of 50% *w/w* with distilled water was made from all of the honey samples. Diluted honey (2 mL) was measured into a 20-mL SPME vial containing a glass covered stirrer. All vials were capped with PTFE-silicon septa and placed in an autosampler tray at room temperature. For SPME, 30/50 µm DVB/CAR/PDMS Stableflex 2-cm long fiber from Supelco (Bellefonte, PA) was used. Samples were brought one-by-one into the magnetic stirring chamber for volatile extraction using a method described in the next section. After the extraction process the fiber was injected into the GC

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inlet for desorption for 10 minutes (either GC–MS (TOF) or GC–O), followed by oven temperature program described in the next section.

2.5. Analysis of odor-active compounds using GC–MS and GC–O

The GC column for both GC-MS (Agilent 6890) and GC-O (Agilent 7890) was DB5-MS, 30 m × 0.25 mm × 1.0 µm (Restek, Bellefonte, PA). GC-MS inlet was PTV, GC–O inlet was split/splitless using Merlin Microseal (Agilent, Santa Clara, CA), and both were run in splitless mode. A 0.75-mm i.d. liner at 250 ºC was used in both injectors. The carrier gas was He, 1.0 mL/min in GC-MS and 2.0 ml/min in GC-O. The GC-MS was equipped with time-of-flight detector (Waters, Manchester, UK) and the GC–O was equipped with odor detection port (Gerstel, Mülheim an der Ruhr, Germany). For the GC-MS data analysis NIST05 library was used. The SPME extraction time and temperature for best sensitivity were chosen based on a previous study (Seisonen et al., 2015). Pre-incubation time 5 min, incubation temperature 60 ºC and extraction time 20 min (250 rpm) and desorption time 10 min were chosen as optimum. The oven program for GC-MS was the following: from 35 ºC, 45 ºC/min to 85 ºC, 9 ºC/min to 200 ºC, 45 ºC/min to 280 ºC holding time 1 min (total 16.67 min). Oven program for GC-O was the following: starting at 35 °C, 17 °C/min to 280 °C, holding time 4 min (total run time 17.41 min). Three trained assessors (female) of age 22–29 years (mean 25 ± 3.6) were used for the GC-O analysis. Each assessor sniffed the samples in duplicate. Posterior intensity method was used with a 5-point scale. For identification of the odor-active compounds, the results of GC-MS and GC-O were correlated using Kovats retention indices.

2.6. NMR spectroscopy

The NMR data was acquired at 298 K using a Bruker Avance-III 600 MHz NMR spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) equipped with a Prodigy TCI cryoprobe and a SampleJet. The honey (100 mg) samples, diluted with Milli-Q water and containing 10% Chenomx internal standard in D_2O were analyzed using a noesypr1d pulse program, as described by Kortesniemi et al. (2016).

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2.7. Data analysis

GC–O data were processed using modified frequency formula (1):

$$MF(\%) = \sqrt{F(\%) * I(\%)} \qquad (1)$$

where *F* (%) is the detection frequency expressed as percentage of maximum detection $\left(\frac{number\ of\ detections}{6} \times 100\%\right)$ and *I* (%) is a sum of odor intensities expressed as percentage of maximum sum of intensity $\left(\frac{sum\ of\ intensities\ from\ 6\ sniffings}{30} \times 100\%\right)$. *MF* (%) shows the importance of the compound in a sample.

The attributes rated on a scale were analyzed using one-way ANOVA and Tukey's post hoc test ($p < 0.05$) and the frequencies for the most and the least liked samples were analyzed using Cochran's Q-test and McNemar's test with IBM SPSS Statistics 24 (SPSS, Inc., Chicago, IL). The results of CATA method including attributes from 12 main sensory categories together with rated attributes and the hedonic liking results were analyzed with principal component analysis (PCA). Partial least squares regression (PLSR) analysis was carried out, to investigate the correlations between sensory attributes and volatile composition. Responses were grouped according to the PCA results prior to running PLSR analysis on groups of highly correlated sensory attributes. For the analysis, only the predictors with variable importance to projection (VIP) value larger than 1 were included. All the data was autoscaled prior to statistical analysis. The PLSR analysis was carried out using R 3.4.0 package "*plsdepot*".

The NMR spectra were subject to line broadening (0.5 Hz), binning (0.02 ppm), normalization (to standardized area) and Pareto scaling prior to multivariate analysis by PCA (SIMCA-P+ v12.0; Umetrics AB, Sweden).

3. Results and discussion

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3.1. Botanical origin of honey samples

The botanical origin of the honey samples was confirmed with melissopalynology and ^{1}H NMR spectroscopy. The relative proportions of pollen types present in the honey samples are shown in supplementary **Table S2**. Pollen analysis is recognized often as an ambiguous yet common method in evaluating the true botanical origin of honey but it still gives an overview of the biotope in the vicinity of the hive. For normally represented pollen, the limit for varietal representativeness is > 45%, relative to total pollen (Von der Ohe et al., 2004). As is typical of Finnish honeys, however, the pollen profiles are highly miscellaneous and the dominant pollen type does not necessarily reflect the accurate botanical origin (Von der Ohe et al., 2004; Salonen et al., 2009). However, the ^{1}H NMR profiles (**Fig. S2**) were in accordance with the declared botanical origins as compared to our earlier data (Kortesniemi et al., 2016) and reference honey samples of our in-house library (not containing sweet clover and willowherb honey). The NMR data were also subject to multivariate analysis (PCA; **Fig. S3**), illustrating the influence of fructose and glucose and specific markers on the variation between the samples.

The buckwheat honey contained mostly pollen from *Trifolium* spp. (33%) and only 19% from *Fagopyrum esculentum*. Markers of buckwheat honey including relatively high levels of isoleucine, leucine, valine and tyrosine were detected with NMR (**Fig. S2** and **Fig. S3**). The cloudberry-bog honey contained pollen from *Vaccinium* spp., *Menyanthes trifoliata*, both *Rubus chamaemorus* and another *Rubus* spp., and *Astralagus alpinus*. Cloudberry-bog honey, including predominantly these bog biotope-related pollen sources is also referred as mire honey (Salonen et al., 2012). As a dioecious plant, only the staminate flower of *Rubus chamaemorus* has pollen but both the staminate and pistillate flowers have nectar. Also, the staminate plants are generally more abundant than the pistillate plants. Therefore, the cloudberry pollen can be considered under-represented in relation to the nectar. The complex NMR profile of the cloudberry-bog honey, now reported for the first time, matched that of our reference sample of the same botanical origin. The final identification of the

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markers will be published separately. Still, the PCA loadings (**Fig. S3D**) revealed that acetic and formic acids are among the strong markers for cloudberry-bog honey when variables of the sugar region (3.09–5.51) are excluded from the model.

The dominating pollen types in the lingonberry honey were *Vaccinium* spp. (37%) and *Trifolium* spp. (36%). NMR marker signals for lingonberry honey and matching the respective reference honey were present at δ 8.51, 8.61 and 8.95 ppm (**Fig. S2A**). As no prior NMR data were available for sweet clover or willowherb honey, the NMR analysis was not conclusive. The pollen of sweet clover was present in low proportion (*Melilotus* spp., 3%) as typical, the NMR profile mostly resembled that of the smooth multifloral honey. We can state that the sweet clover was not dominating botanical origin, based on the pollen and NMR profiles, although a faint cinnamon aroma (coumarin, specific for sweet clover) was observed and the apiary was in the proximity of flowering sweet clover. The under-representation of *Epilobium* pollen (approx. 3%) in Finnish willowherb (also known as fireweed) honey is consistent with prior knowledge (Salonen et al., 2011). Instead, the dominating pollen in willowherb honeys belong to Rosaceae and *Trifolium* (Salonen et al., 2011) as was found in our study (**Table S2**). Leucine, turanose and δ 5.40 ppm (maltose/sucrose) were relatively high in the willowherb honey profile, while the region δ 6.0–10.0 ppm practically lacked signals compared to other samples (**Fig. S2**).

The main pollen types in the granular multifloral *Rubus* spp. *Trifolium* spp., *Vaccinium* spp. and Brassicaceae, whereas in the smooth multifloral honey Brassicaceae, *Trifolium* spp., *Rubus* spp., *Salix* spp., and *Vicia faba*, dominated. The multifloral honeys were characterized by a lack of obvious markers in the NMR profiles (**Fig. S2**).

3.2. Sensory analysis

The rated attributes (on a scale 1–5) and the most and least preferred samples (%) are shown in **Table 1**. The most frequent attributes (used by >10% of the panel/sample) are shown in

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Supplementary material **Table S3**. Due to the use of voluntary and untrained consumers in the sensory test, "attribute in general", "no attribute" and "I don't know" attributes were included (**Table S3**). In general, "attribute in general" and "no attribute" were the most frequently used attributes, showing that panelists had difficulties in distinguishing the respective odor or flavor attribute, thus selecting the "attribute in general" instead or, on the other hand, they were able to decide if a sample is missing a certain attribute ("no attribute"). Moreover, only a few panelists correctly selected the attributes "lingonberry" or "cloudberry" for the corresponding samples (**Table S3**).

The data in **Tables 1** and **S3** were used in PCA models (**Fig. 1A–D**). Due to large number of variables, four different PCA models were made. **Fig. 1A,** where 87% of the variance is in the first two components, contains the odor and flavor attributes rated on a scale from **Table 1**. Overall odor and flavor intensities were the highest in buckwheat and cloudberry-bog honeys, which were also the least liked samples (the lowest frequency in the most preferred and highest in the least preferred). Those samples also had the highest intensities of sourness. The relatively high levels of organic acids (**Fig. S3C–D**) in cloudberry-bog honey are likely contributors to its perceived acidity (**Fig. 1A**). However, the relatively high fructose content (**Fig. S3A–B**) and thereby the sweetness (**Fig. 1A**) may mask some of the sourness.

Granular multifloral honey was characterized by high sweetness and low acidity whereas smooth multifloral honey was perceived as the most familiar honey in terms of both odor and flavor, as expected (**Table 1**, **Fig. 1A**). The amounts of fructose and glucose in honey reflect the botanical origin, sweetness and texture in terms of fluidness/hardness and crystallization (Bogdanov, Ruoff & Persano Oddo, 2004; Salonen, 2012; da Silva et al., 2016). The granular texture of honey is explained by the crystallization of glucose, while liquid honeys have a relatively high content of fructose. The granular and smooth multifloral honeys and the willowherb honey were deemed the sweetest (**Table 1**). The sweetness and the relatively high fructose content (**Fig. S3A–B**) of the

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willowherb honey were consistent with former studies (Salonen et al., 2011; Salonen, 2012). At the time of evaluation, the willowherb honey showed some signs of crystallization (possibly due to the storage conditions or heterogenous honey composition).

The sensory quality of honey when perceived as familiar was generally favored over the more unfamiliar, strongly aromatic varietal honeys. According to a study by Swanson and Lewis, mild honeys, such as mixed-flower and fireweed honeys were regarded as the most acceptable by the panelists evaluating premium honeys (Swanson & Lewis, 1991–1992).

In the PCA model in **Fig. 1B** (73% of variance in the first two components) the two least preferred samples were well characterized and clearly separated from the other samples. Buckwheat honey located on the upper left side of the correlations loadings plot was characterized by earthy (e.g., "mushroom", "wet earth"), microbiological ("yeasty") and animal-like (e.g., "barnyard") attributes as well as missing the berry-like or fruity attributes. Cloudberry-bog honey located on the lower left side of the plot had herbaceous, woody, and chemical flavor notes and it was described with brown sugar-like flavor. The other more preferred samples were located on the right side of the plot with "caramel", "fruity", "berry-like" and "floral" attributes. The aroma of lingonberry honey has been described earlier with the terms "toffee-like", "citrus-like" and "fruity" (Salonen & Julkunen-Tiitto, 2012). Willowherb honey has been previously characterized as very weak, "hay-like" and "malty" (Salonen et al. 2011). In this study, the willowherb honey had very low odor and flavor intensities lacking the majority of the attributes (i.e. highest frequencies in many of the "no attribute" variables in **Table S3**). In addition to their unique aroma and flavor, Finnish buckwheat and willowherb honeys have shown significant antimicrobial activity against human-pathogenic streptococci and staphylococci (Huttunen, Riihinen, Kauhanen, & Tikkanen-Kaukanen, 2012).

In the model in **Fig. 1C** representing appearance attributes, 72% of the variance in the data is shown in the first two components. Buckwheat and cloudberry-bog samples had the highest intensity of

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color (**Table 1**) with "brown" and "orange" attributes in the PCA model. The intense dark color of a honey can be linked to its radical-scavenging capacity (González Lorente et al., 2008). However, in this study the two darkest honeys were the least preferred among the samples therefore probably the least likely to be chosen by a consumer. Willowherb honey has previously been characterized with very light color, which makes it distinguishable from the other Finnish honeys (Salonen et al., 2011). In the PCA model in **Fig. 1D** (82% of variance in the first two components) the textural attributes from CATA were used as X-variables. In general, the least liked samples had the largest crystals (upper left in the plot) or were most liquid (upper right). Color and textural attributes can generally be significant factors for consumers in differentiating various honeys (González-Viñas, Moya, & Cabezudo, 2003).

3.3. GC-O analysis and correlation with sensory data by using PLSR

In GC-O analysis in total 73 odor-active compounds were found, from which 37 were common in all the analyzed samples. **Table 2** shows the calculated modified frequency values of each compound in the analyzed honey samples. The absence of a specific compound in some of the samples means that it was not detected with GC-O; it still might occur in the sample but in concentration below detection limit of GC-O assessors. GC-MS data were used for tentative identification of the compounds.

PLSR was applied on groups of correlated attributes, resulting in four biplots in total (**Fig. 2**). The attribute "caramel" was left out from the analysis as it did not result in a satisfactory model even when modelled alone (PLSR1). For the other four groups R^2Y values with two components varied from 0.82–0.97 and Q^2 value from 0.55–0.80 and with 5 components Q^2 values were between 0.82–0.97.

The sum of modified frequencies of all the compounds in the honey samples showed good correlation with the results of sensory intensities. Buckwheat, cloudberry-bog and smooth

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multifloral honey samples had the highest total modified frequency values. Buckwheat and cloudberry-bog honey were also evaluated the highest in terms of the odor intensity and all three abovementioned samples got the highest scores in flavor intensity category. At the same time willowherb and crystallized multifloral samples gained the lowest values in terms of odor and flavor intensity and also total modified frequency value.

The most important odor-active compounds in buckwheat honey were 3-methylbutanal, butanoic acid, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone and phenylacetaldehyde. 3-Methylbutanal is believed to be the main compound responsible for the characteristic malty flavor of buckwheat honey (Panseri et al. 2013, Pasini et al. 2013, Zhou, Wintersteen, & Cadwallader 2002). It has been suggested that the unpleasant aroma of the buckwheat honey may originate from compounds such as butyric acid (cheese- and fecal-like) and *p*-cresol (cow- and barn-like) (Zhou et al., 2002). The modified frequencies of *p*-cresol and butyric acid were one of the highest (71 and 89, respectively) in the buckwheat honey. According to PLSR analysis in **Fig. 2**, 3-methylbutanal (V5) and *p*-cresol (V33) have a strong correlation with animal-like, microbiological and earthy aroma. Also, 1-heptanol (V22), methional (V19) and 2-methyl-2-pentanol (V9) have a very strong correlation with the abovementioned attribute group. Buckwheat honey had the highest values in the cases of dimethyl sulfide (sulfur), 2-methyl-2-pentanol (cheesy) and unknown animal-like compound (RI = 1123), which all may cause unpleasant notes, and phenylacetaldehyde (honey-like), which has been claimed previously to contribute to the typical buckwheat honey flavor profile (Pasini et al. 2013, Zhou et al. 2002).

Cloudberry-bog honey had the highest values of 1-propanol (pungent), *p*-cymene (solvent), isophorone (herbal) and citral (citrus), which explain well the woody, herbal and chemical notes recognized in sensory analysis. All the above-mentioned compounds (V2, V29, V36) had very strong correlation with woody and also herbal and chemical notes. In addition, methional (V19, potato) and (*Z*)-oak lactone (V70, aniseed) revealed a high positive correlation with herbal notes

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according to PLSR analysis. Also, the relatively high number of different compounds present in cloudberry-bog honey in remarkably higher intensities compared to the other samples, support the statement that cloudberry-bog honey is the most aromatic honey analyzed in this study.

The aroma of lingonberry honey was described as pleasant and sweet, with notes of vanilla and caramel. In the GC–O analysis vanillin and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (caramel) were the most abundant odor-active components. Lingonberry honey could also be characterized by a relatively higher content of ethyl 3-phenylpropanoate (floral).

Sweet clover honey had the highest phenylacetic acid (honey-like) and (*Z*)-3-nonenal (cucumber) contents. Also, it could be characterized by the absence of 3-methylbutanal (malty). Willowherb honey has the fewest different odor-active compounds and none of them differentiate it from the other honey samples. The most intensive compounds were 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (caramel), (*E*)-2-nonenal (cucumber), hexyl hexanoate (apple) and (*E*)-*β*-damascenone (cooked apple).

Crystallized multifloral honey had higher values of different compounds with fruity notes, like 2,3-butanediol, ethyl-3-methylbutanoate and ethyl hexanoate. According to PLSR analysis, the perception of fruity notes is strongly influenced by the absence of typical animalic and earthy notes together with intense honey-like flavor.

Brassicaceae pollen was predominant in the smooth multifloral honey (**Table S2**). The aroma of rape (*Brassica napus*) honey was studied by Ruisinger and Schieberle (2012). The most important odor-active compounds were identified as (*E*)-*β*-damascenone (cooked apple-like), phenylacetic acid (honey-like), 4-methoxybenzaldehyde (aniseed-like), 3-phenylpropanoic acid (floral, waxy), 2-methoxy-4-vinylphenol (clove-like) and phenylacetaldehyde (floral) (Ruisinger & Schieberle, 2012). Most of the above-mentioned compounds were also present in smooth multifloral honey in significant concentrations. Also, isophorone which has been claimed to indicate the floral origin

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within the heather family was present in smooth multifloral honey (Guyot, Scheirman, & Collin, 1999; Seisonen et al. 2015).

3.5. Honey consumption, consumer habit and preference

At the end of the sensory evaluations, panelists were asked various questions related to their usage and preferences of honeys. Several cluster analysis models (two-step clustering) were created, in order to study their impact on the most and least preferred samples (Supplementary material **Table S4**). However, clusters based on age, usage ("frequent users" vs "non-users") or honey origin ("local" *vs* "no preference") did not have any impact on choosing the most or least preferred samples.

The panelists generally used honey either several times a week (29%, $n = 62$), a few times in a month (27%) or a few times in a year (21%) with an average between "a couple of times a month" (value 3 = mean on a scale from 1 to 7), and "Once a month" (value 4). The most common ways of usage of honey were in hot drinks (answer selected by 73% of panelists in **Table S4**), in cooking (53%) and in direct consumption (52%). The panel evaluated the flavor/taste (76%) to be the most important factor for making buying decision. Other important characteristics were consistency (65%) and origin (55%). For example, liquid honey was preferred by 60% and honey from a certain country, e.g., home country, by 57%. Only 18% preferred monofloral/varietal honeys. This may be due to the limited availability, generally higher prices and/or the unfamiliarity of these specialty honeys. The familiarity of the product was not regarded as among the most important characteristics in honey (21%) but still the familiarity of the flavor and odor showed positive correlation with most preferred samples (**Fig. 1A**). In a study by Stolzenbach et al. (2013) familiarity was positively linked to the local honeys whereas novel honeys containing mixtures of other fruit materials in the honeys were considered as "too" novel for consumers, resulting in negative emotional responses (Stolzenbach, Bredie, & Byrne, 2013).

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4. Conclusion

Multifloral honeys were perceived as highly familiar and liked by the panelists. In addition to the smooth multifloral honey, sweet clover and lingonberry honeys received the highest scores in liking. The honeys with strong and unfamiliar odor, flavor and aftertaste, dark color, and negative correlation to perceived sweetness (buckwheat and cloudberry-bog) were the least preferred. Both honeys were also described with strongly negative and unpleasant attributes. In general, flavor and consistency were important characteristics in honey, steering the consumer's choice. The attributes that were considered unfamiliar negatively affected the pleasantness and liking of honey. Overall, describing the honey attributes was challenging to the untrained panelists.

By using PCA and PLSR, sensory notes characteristic to specific honey samples were found and correlated with the odor-active compounds determined with GC–O. The two least preferred samples (buckwheat and cloudberry-bog) were differentiated from the others the most. Buckwheat honey was characterized by earthy (e.g., "mushroom", "wet earth"), microbiological ("yeasty") and animal-like (e.g., "barnyard") notes which had the strongest positive correlations with specific volatile compounds like 3-methylbutanal and *p*-cresol. According to PLSR, "fruity" category had a strong negative correlation with the compounds responsible for the above-mentioned categories. Cloudberry-bog honey could be characterized with herbaceous, woody and chemical notes, which were highly correlated to the presence of 1-propanol (pungent), *p*-cymene (solvent), isophorone (herbal) and citral (citrus).

The study provides valuable information on the sensory characteristics of different honey types and on the honey consumption, consumer habits and preference in Finland. The data add to the knowledge base of the effects of the botanical origin on the sensory-chemical profiles of Finnish honeys, especially on the odor-active compounds. The varietal honeys included in the study were relatively unknown to consumers and generally disliked. Further studies are needed, in order to

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cover a wider selection of honey samples (of the same and additional botanical origins) and to obtain sensory profiles with greater detail (by using a trained panel).

Conflicts of interest

None.

Acknowledgments

The Finnish Cultural Foundation and the University of Turku Graduate School's Doctoral Programme in Molecular Food Sciences (UTUGS/DPMLS) are gratefully acknowledged for the financial support. We thank Heikki Vartiainen (Finnish Beekeepers' Association) and Lassi Kauko for assistance in selecting the honey samples. Finally, we thank all the panelists for their contribution.

Appendix A

Supplementary material.

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Figure captions

Figure 1. Principal component analysis models showing interactions among sensory variables (blue font) and most and least preferred variables (red font) in seven honey samples (green font). A) Rated odor and flavor attributes (*X*-variable $n = 9$), B) odor and flavor CATA variables ($n = 76$), C) appearance variables ($n = 10$), and D) texture variables ($n = 11$). Attributes from Table 1 and Supplementary material Table S3 (attribute "I don't know" excluded). Sample abbreviations: buckwheat, BW; cloudberry-bog, CBB; lingonberry, LB; sweet clover, SC; willowherb, WH; multifloral granular, MFG; multifloral smooth, MFS.

Figure 2. Partial least squares regression analysis, showing correlation between sensory overall categories (orange font) and volatile components determined by GC–O (blue font). A) Correlation biplot for nutty and spicy categories. B) Correlation biplot for berry-like, floral and fruity categories. C) Correlation biplot for herbaceous, woody and chemical categories. D) Correlation biplot for earthy, microbiological and animalic categories.

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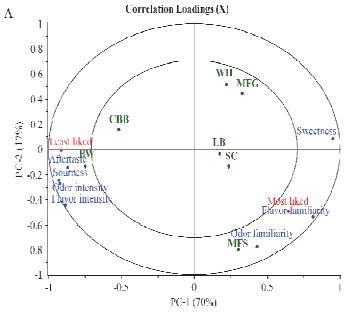

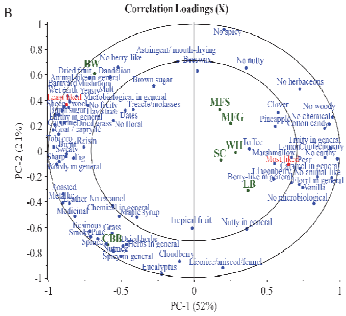

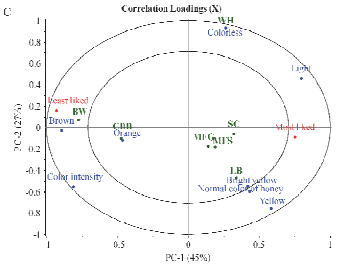

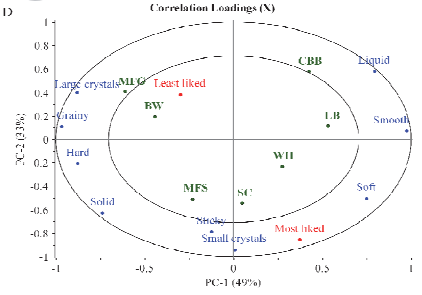

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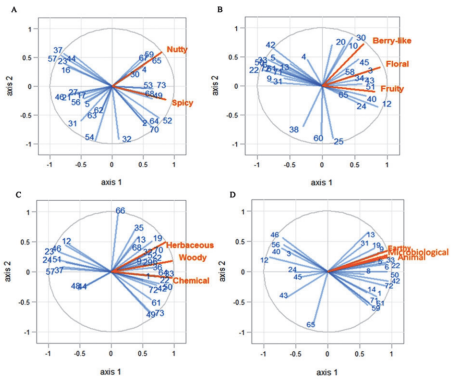

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Table 1. Attributes rated (scale 1–5) and the most and least preferred samples (%) by the panel (n = 62).

attribute[a]	honey samples[b]						
	BW	**CBB**	**LB**	**SC**	**WH**	**MFS**	**MFG**
color intensity	4.0 ± 0.6a	3.7 ± 0.5b	2.5 ± 0.6d	2.5 ± 0.6cd	1.1 ± 0.3e	2.8 ± 0.6c	2.6 ± 0.7cd
odor intensity	2.5 ± 1.2b	2.2 ± 1.1b	2.6 ± 1.0b	2.5 ± 1.2b	2.3 ± 1.1b	3.5 ± 1.1a	2.7 ± 1.1b
odor familiarity	2.1 ± 1.0c	2.0 ± 1.0c	3.2 ± 0.9b	3.5 ± 1.2ab	2.5 ± 1.1c	3.8 ± 1.1a	3.1 ± 1.3b
flavor intensity	4.2 ± 0.6a	4.0 ± 0.6ab	3.3 ± 0.6cde	3.4 ± 0.6cd	3.1 ± 0.6de	3.6 ± 0.6bc	2.9 ± 0.6e
flavor familiarity	2.1 ± 1.0c	2.0 ± 1.0c	3.2 ± 0.9b	3.5 ± 1.2ab	2.5 ± 1.1c	3.8 ± 1.1a	3.1 ± 1.3b
sweetness	3.2 ± 0.6b	3.2 ± 0.6b	3.7 ± 0.6ab	3.6 ± 0.6ab	3.8 ± 0.6a	3.7 ± 0.6a	3.7 ± 0.6a
sourness	2.4 ± 0.6a	2.0 ± 0.6ab	1.8 ± 0.6bc	1.7 ± 0.6bc	1.7 ± 0.6bc	1.8 ± 0.6bc	1.6 ± 0.6c
aftertaste intensity	3.7 ± 0.6a	3.9 ± 0.6a	3.1 ± 0.6b	2.9 ± 0.6b	3.0 ± 0.6b	2.9 ± 0.6b	2.2 ± 0.6c
most preferred (%)	1.6b	4.8b	19.4a	25.8a	14.5ab	22.6a	3.2b
least preferred (%)	56.5a	21.0b	0.0c	1.6c	8.1bc	1.6c	3.2c

[a] Included only the rated attributes (average ± standard deviation); results of Check-All-That-Apply are shown in **Supplementary Table S3**. Statistically significant differences are shown with letters a–e based on one-way ANOVA and Tukey's post hoc test ($p < 0.05$) for the rated attributes and Cochran's Q-test and McNemar's test for frequency data.

[b] Sample abbreviations: buckwheat, BW; cloudberry-bog, CBB; lingonberry, LB; sweet clover, SC; willowherb, WH; multifloral smooth, MFS; multifloral granular, MFG.

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Table 2. Compounds and their modified frequencies according to GC–O.

	compound	Kovats RI[a]	observed RI	ident.[c]	flavour description	BW	CBB	LB	SC	WH	MFG	MFS
1	dimethyl sulfide	505	516	St, MS, L	sulfur	49	35	39	24	29	13	0
2	1-propanol	536	541	L,	pungent	0	57	21	11	0	0	29
3	2,3-butanedione	593	599	St, L,	butter	28	41	49	49	24	41	47
4	acetic acid	600	616	L	vinegar	42	38	47	39	45	22	18
5	3-methylbutanal	650	662	MS, L	malty	82	41	39	0	37	9	61
6	methyl 2-methylpropanoate	685	674	L	floral	28	13	11	0	0	0	0
7	methyl thiocyanate	685	686	L	roasty, onion	0	38	0	39	0	0	71
8	3-pentanol	759	760	L	fruity	32	13	24	18	11	15	0
9	2-methyl-2-pentanol	768	765	L	cheese	47	28	13	32	22	18	21
10	1-hexen-3-ol	789	778	L	grass	0	13	21	0	0	0	15
11	(*Z*)-2-penten-1-ol	783[b]	784	L	plastic	42	44	21	53	47	52	24
12	2,3-butanediol	806	805	MS, L	fruity	0	18	54	53	18	71	47
13	butyric acid	820	824	St, MS, L	cheese	89	87	86	88	84	85	88
14	ethyl 2-methylbutanoate	846	838	L	apple	58	49	52	54	52	46	39
15	ethyl 3-methylbutanoate	854	853	MS, L	fruity	68	41	57	68	58	75	29
16	methyl 2-(methylthio)acetate	894	883	L	roasty	73	63	70	68	68	65	73
17	ethyl pentanoate	900	902	MS, L	fruity	33	0	32	0	0	18	0
18	heptanal	903	909	L	fat	0	57	0	0	34	47	29
19	methional	909	920	St, L	potato	39	33	26	29	11	13	26
20	unknown 1		930		cheese	52	69	58	73	21	25	75
21	methyl hexanoate	934[b]	937	L	fruity	11	0	0	0	0	0	37
22	1-heptanol	962	955	L	green	42	28	0	11	15	0	13
23	1-octen-3-ol	982	983	St, L	mushroom	73	44	77	77	63	77	75
24	1-octen-3-one	976	986	L	metal	77	69	84	86	80	82	77
25	ethyl hexanoate	1002	996	MS, L	fruity	0	0	0	0	0	28	0
26	methyldihydrothiophenone	998	998	L	roasty	84	80	82	85	49	77	88
27	unknown 2		1011		hay	0	0	0	0	0	0	55
28	α-phellandrene	1007	1013	MS, L	herbal	65	38	58	53	75	35	55

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	compound	Kovats RI[a]	observed RI	ident.[c]	flavour description	BW	CBB	LB	SC	WH	MFG	MFS
8												
2	*p*-cymene	1027	1032	MS, L	solvent	0	72	0	15	0	0	71
9												
3	D-limonene	1030	1058	MS, L	mint	13	25	34	37	21	0	35
0												
3	phenylacetaldehyde	1049	1065	St, MS, L	honey	91	80	84	68	71	80	82
1												
3	(*Z*)-linalool oxide	1070	1071	MS, L	floral	26	46	24	7	11	41	24
2												
3	*p*-cresol	1075	1077	L	urine	71	63	24	21	32	9	49
3												
3	(*Z*)-3-nonenal	1096	1098	MS, L	cucumber	0	0	0	24	0	0	18
4												
3	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	1107	1102	L	caramel	89	89	89	89	86	87	89
5												
3	isophorone	1117[b]	1112	L	herbal	0	89	0	0	0	0	60
6												
3	unknown 3		1123		animal	86	0	62	73	65	75	69
7												
3	2-phenylethyl alcohol	1118	1125	St, MS, L	flower	28	15	0	24	11	33	21
8												
3	unknown 4		1138		malty, sour	53	35	0	75	32	15	71
9												
4	lilac alcohol B	n.a	1144	MS, L	honey, floral	0	15	26	14	0	31	26
0												
4	(*E*,*E*)-2,6-nonadienal	1162[b]	1152	St, MS, L	green	13	25	21	7	34	13	21
1												
4	lilac aldehyde A	1154[b]	1158	MS, L	flowery	37	31	26	21	26	13	24
2												
4	(*E*)-2-nonenal	1162	1162	MS, L	cucumber	37	69	69	68	86	63	82
3												
4	2-phenylethylthiol	1176	1168	L	rubber	86	49	77	71	77	59	77
4												
4	ethyl benzoate	1185	1184	MS, L	honey	0	71	63	32	29	35	68
5												
4	unknown 5		1195		roasty	39	28	51	47	34	69	60
6												
4	(*E*)-linalool oxide	1212	1202	MS, L	herbal	49	0	7	49	18	0	67
7												
4	isobutyric acid	1215	1213	L	dry	13	0	32	34	49	13	80
8												
4	benzothiazole	1240	1230	L	flowery	43	72	58	39	68	35	47
9												
5	ethyl phenylacetate	1252	1239	St, L	honey	89	85	69	73	77	63	75
0												
5	phenylacetic acid	1262	1250	L	honey	21	0	37	73	39	25	47
1												
5	citral	1254	1258	L	citrus	11	72	34	37	24	38	26
2												

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	compound	Kovats RI[a]	observed RI	ident.[c]	flavour description	BW	CBB	LB	SC	WH	MFG	MFS
53	D-carvone	1265[b]	1267	L	thyme	77	80	79	77	71	77	52
54	*p*-anisealdehyde	1275[b]	1283	L	aniseed	56	67	61	11	26	59	62
55	γ-butyrolactone	1299	1301	L	honey	49	9	18	58	47	55	0
56	unknown 6		1305		metal	0	0	13	11	0	69	21
57	3-phenylpropanoic acid	1321	1323	L	herbal	86	41	77	81	77	82	84
58	ethyl 3-phenylpropanoate	1351	1354	L	floral	0	9	56	0	0	15	0
59	unknown 7		1360		cellar	65	59	49	66	71	0	26
60	eugenol	1364	1368	MS, L	clove	52	57	49	48	62	72	52
61	hexyl hexanoate	1379	1385	MS, L	apple	89	88	77	24	80	36	17
62	*E*-β-damascenone	1386	1400	St, L	apple	80	76	88	47	87	75	73
63	vanillin	1410	1420	St, L	vanilla	80	76	88	47	85	75	73
64	unknown 8		1430		dill	11	59	45	0	11	0	13
65	geranyl acetone	1448	1448	L	herbal	11	25	34	34	52	25	0
66	β-caryophyllene	1467	1466	MS, L	woody	52	58	49	68	26	59	49
67	ethyl cinnamate	1460[b]	1484	L	cinnamon	73	69	66	73	73	69	58
68	ethyl laurate	1493	1494	L	dill	32	72	21	66	32	51	45
69	methyl dodecanoate	1509	1513	MS, L	dill	29	0	21	24	11	0	18
70	(*Z*)-oak lactone	1538	1537	L	aniseed	0	38	21	11	0	13	7
71	hexyl octanoate	1566	1559	L	peppermint	68	57	62	32	66	44	42
72	(*E*)-whiskey lactone	1629	1633	L	chamomile	26	15	13	0	11	0	0
73	γ-dodecalactone	1685	1687	L	herbal	7	21	0	0	21	0	0
	total modified frequency					**2946**	**3047**	**2893**	**2784**	**2636**	**2590**	**3070**

[a] www.flavornet.org. [b] www.pherobase.com [c] Basis for identification: St, standard compound; MS, GC–MS; L, literature (RI and occurrence in honey).

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Highlights

- Sensory profiles of seven Finnish honeys were correlated with GC–MS/O data.
- Seventy-three odor-active compounds were detected in the honey samples with GC–O.
- Sweet and mild (multifloral) honeys are generally preferred and familiar.
- Buckwheat and cloudberry-bog honeys with intense sensory properties were not liked.

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