

Astrid Sarapa¹, Leonhardt A. F. Wagner¹, Helene Loos², Andrea Buettner²

¹Chair of Aroma and Smell Research, Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany

²Chair of Aroma and Smell Research, Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany; Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany; FAU Research Center "New Bioactive Compounds" (FAU NeW), Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany

Propolis, a complex resinous material produced by honeybees (*Apis mellifera*), has attracted considerable attention due to its various bioactive properties and potential applications. This study represents a novel investigation of the aroma-active compounds in propolis samples from two distinct geographical and climatic regions: Croatia and Bavaria. The samples were collected in the summers of 2023 and 2024 in four climatic zones: Alpine Bavaria, Continental Croatia, Alpine Croatia and Mediterranean Croatia.

The aroma-active compounds were identified using gas chromatography-olfactometry (GC-O), gas chromatography-mass spectrometry (GC-MS) and two-dimensional gas chromatography-mass spectrometry/olfactometry (2D-GC-MS/O). The advanced analytical techniques were complemented by reference standards to ensure accurate identification of the compounds. To further define the sensory profile of propolis, a trained panel from the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Faculty of Sciences, Department of Chemistry and Pharmacy, Chair of Aroma and Smell Research, conducted sensory evaluations using an in-house flavour language.

Samples were pulverised using liquid nitrogen, then 0.5 g was extracted in 100 ml dichloromethane (DCM). The extracts were filtered, prepared for solvent assisted flavour evaporation (SAFE) apparatus and concentrated to 100 µl for analysis. An odour extract dilution analysis (OEDA) was conducted using the DB-FFAP column to determine the potential impact of individual odour-active compounds on the overall aroma of propolis samples. The flavour dilution factors (FD) were determined through stepwise dilution of the undiluted distillate with DCM at a ratio of 1:5 (v/v). In the proposed presentation, we will highlight the main substances and sensory impressions that were found to be characteristic to all propolis samples, such as the vanilla-like attribute that correlates with the substance vanillin, but will also aim at elaborating sensory differences between samples and the respective causative substances.

To the best of our knowledge, this study is the first comparison of aroma-active compounds in propolis samples from Croatia and Bavaria. By examining the impact of climatic and geographical factors on the chemical and sensory properties of propolis, it provides valuable insights into a potential regional divergence in this bee product and on how different plant sources may be shaped by environmental conditions, potentially influencing its chemical composition and thus aroma profile.



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Analysis of aroma-active compounds in propolis from different climate regions in Bavaria and Croatia

Astrid Sarapa, Leonhardt A. F. Wagner, Helene M. Loos, Andrea Buettner

Apis mellifera



History

Important role in different civilizations



Distribution

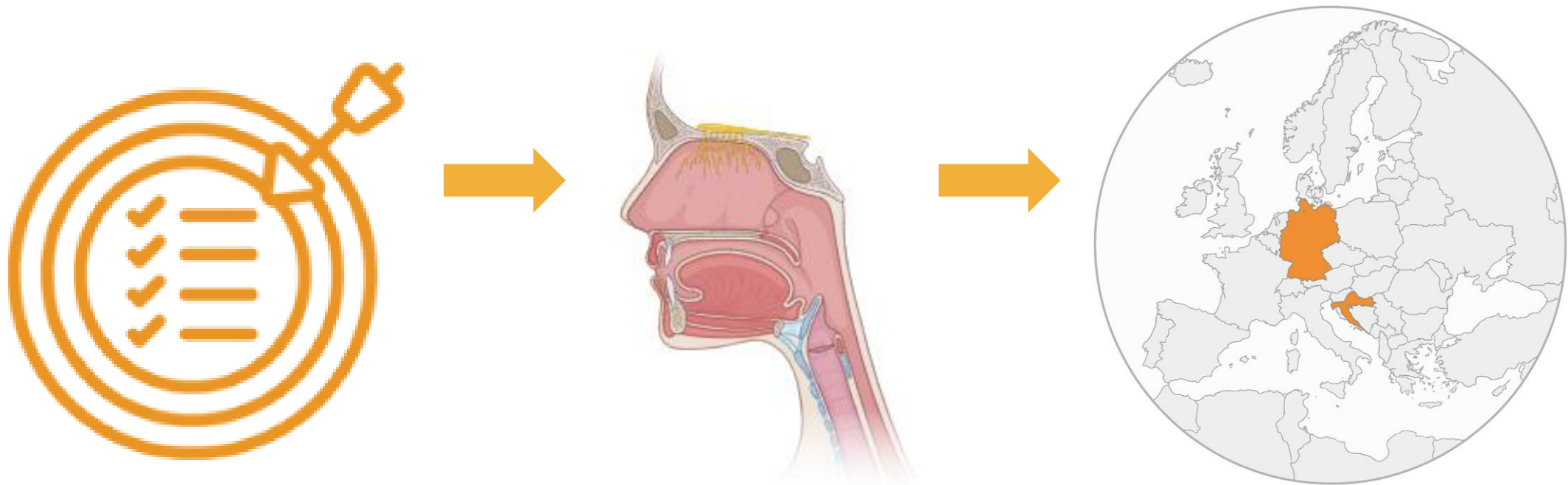
Europe, the Middle East, Africa, Australia, New Zealand, North and South America, Asia



Nature

Important pollinator of crops

Identification of aroma-active compounds in propolis samples from two distinct geographical and climatic regions: Bavaria, Germany and Croatia



Common aroma-active components of propolis from 23 regions of China

Chao Yang,^a Liping Luo,^b Haijing Zhang,^a Xu Yang,^a Yu Lu^a and Huanlu Song^{a*}

Abstract
BACKGROUND: The study of propolis odorants by gas chromatography–olfactometry–mass spectrometry has rarely been put into practice until now. This study aimed to investigate the common odorants of 23 samples of Chinese propolis and analyze the relationship between the principal components and the regions where the samples had been obtained.
RESULTS: By using dynamic headspace sampling, gas chromatography–olfactometry–mass spectrometry and two internal standards, the principal aroma-active components of 23 propolis samples were analyzed qualitatively and quantitatively. The common aroma-active components were compared with each other by principal component analysis. The concentrations, odor activity value (OAV) and sensory evaluation score. The components with high OAVs were acetic acid, 2-phenylethyl acetate, and naphthalene. In decreasing order, which approximated the sensory evaluation score. On the discrete-point map the first three principal components seemed to be grouped into three patterns, while four samples were outliers.
CONCLUSION: The concentrations of aroma-active components were closely related to the regions from a discrete-point map by the first three principal components. It was confirmed that components with high concentrations did not always play important roles in odor contribution, for odor level was linked with their threshold. OAVs could reflect the level of odor exactly according to the approximate result from the comparison between OAVs and sensory evaluation scores.
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Keywords: propolis; aroma-active component; dynamic headspace sampling (DHS); gas chromatography–olfactometry–mass spectrometry (GC–O–MS); odor activity value (OAV); sensory evaluation score; principal component analysis (PCA)

Chen et al.,
China, 2009



- 51 aroma-active compounds
 - 44 identified compounds
 - 4 tentatively identified compounds
 - 3 unknown compounds



Quantitation and Seasonal Variation of Key Odorants in Propolis

Monika Tomaszewski,¹ Melissa Deitz,¹ Ari Novy,^{1,2,3,4} Thomas G. Hartman,¹ Martin Steinhaus,^{1,5} Curtis R. Lockett,¹ and John P. Munafò, Jr.^{1,6}

¹Department of Food Science, Rutgers–The State University of New Jersey, 65 Dudley Road, New Brunswick, New Jersey 08901, United States
²Department of Food Science, University of Tennessee, Knoxville, Tennessee 37996, United States
³San Diego Botanic Garden, 130 Quail Garden Drive, Encinitas, California 92024, United States
⁴Department of Anthropology, University of California San Diego, La Jolla, California 92093, United States
⁵Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20001, United States
⁶Ludwig Institute for Food Systems Biology at the Technical University of Munich, Leo-Mutterer-Str. 34, 85354 Freising, Germany

ABSTRACT: Propolis is a fragrant material produced by bees and is commonly used as an ingredient in food, beverage, and consumer-grade toiletries. Application of a comparative aroma extract dilution analysis (AEDA) to volatiles isolated from propolis over three consecutive years afforded 48 odorants with flavor dilution (FD) factors 2–4, including 21 compounds not previously reported in propolis. Despite differences in FD factors of some compounds, the overall integrated variation in the odorants was low. Compounds with FD ≥ 34 were quantitated by stable isotope dilution assays (SIDs), and odor activity values (OAVs) were calculated. A total of 22 compounds showed OAVs ≥ 1, including (E)-nerolidol (OAV 1700), linalool (OAV 1700), linalyl acetate (OAV 1700), and 3-phenylpropyl acetate (OAV 1700). An odor reconstruction model prepared from deodorized beehives and the 22 odorants in their natural concentrations closely matched the odor profile of authentic propolis. The results of this study will help to establish a basis for future research on the variability of propolis scents from different geographical locations, produced by different bee species, and collected from different botanical sources, all of which are largely unknown.
KEYWORDS: propolis; solvent-assisted flavor evaporation; gas chromatography–olfactometry; aroma extract dilution analysis; stable isotope dilution assay

Tomaszewski et al.,
USA, 2019



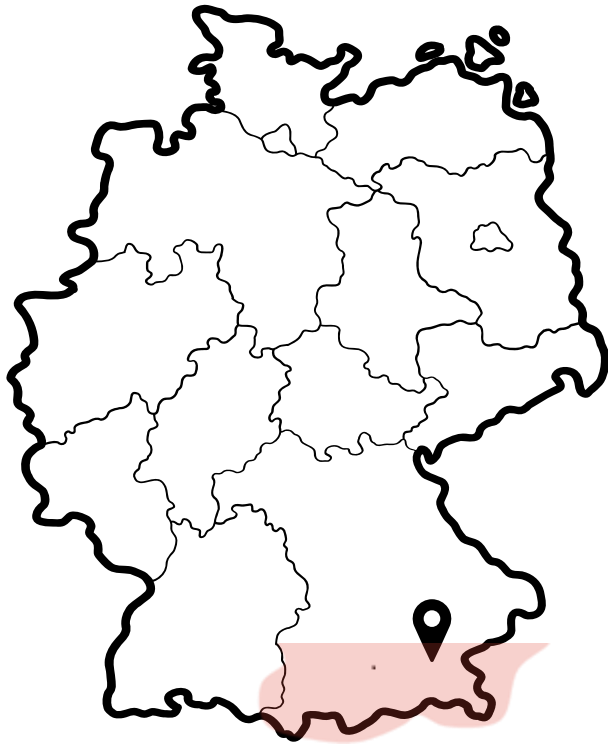
- 48 aroma-active compounds
 - 40 compounds detected in all propolis samples (years 1 through 3)
- 21 compounds not previously reported in propolis

1

Collecting samples - locations: Bavaria, Germany and Croatia

Summer 2023 + summer 2024

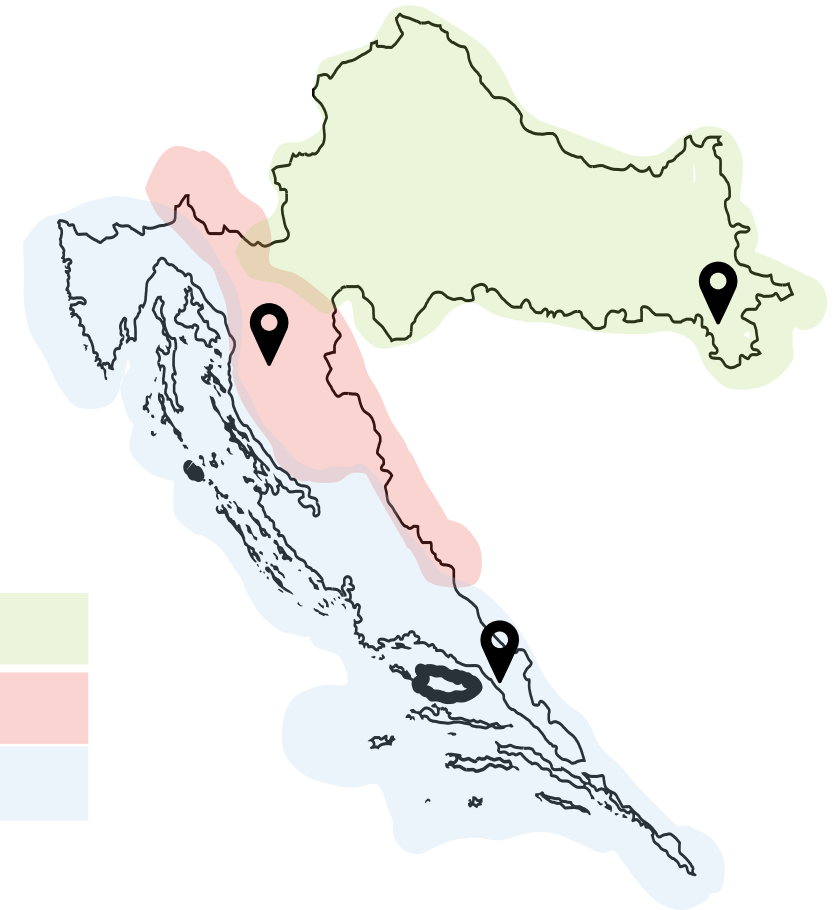
Different climatic regions



Continental

Mountain

Mediterranean



2

Sample preparation

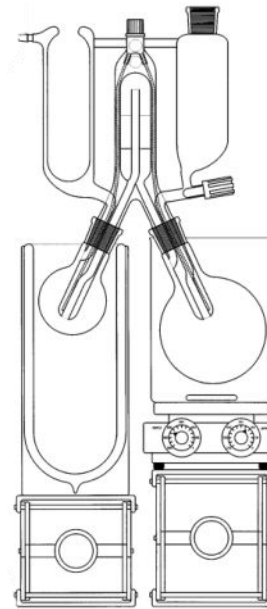
SAFE -solvent-assisted flavor evaporation



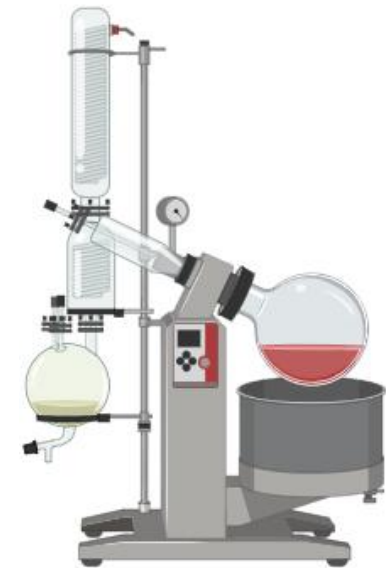
Sample Preparation



Solvent Extraction



SAFE

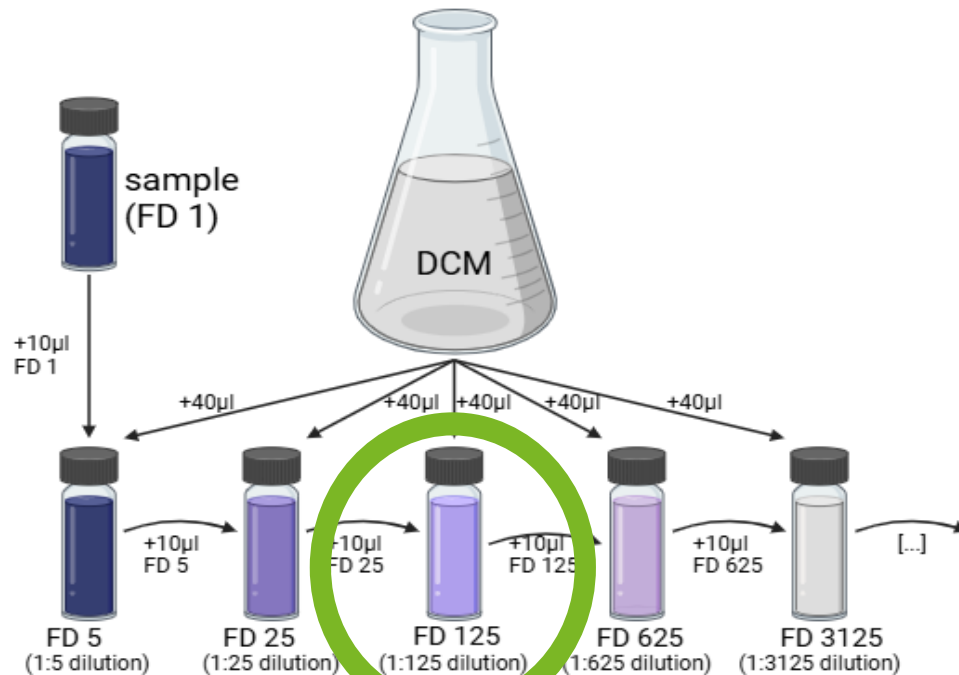


Rotary Evaporation

3

Analysis - Identification

Odour Extract Dilution Analysis (OEDA)

**OEDA**

4

Analysis – Sensory evaluation

Aroma profile of propolis

Sensory evaluations using an in-house flavour language



Covered samples with random 3 digit code

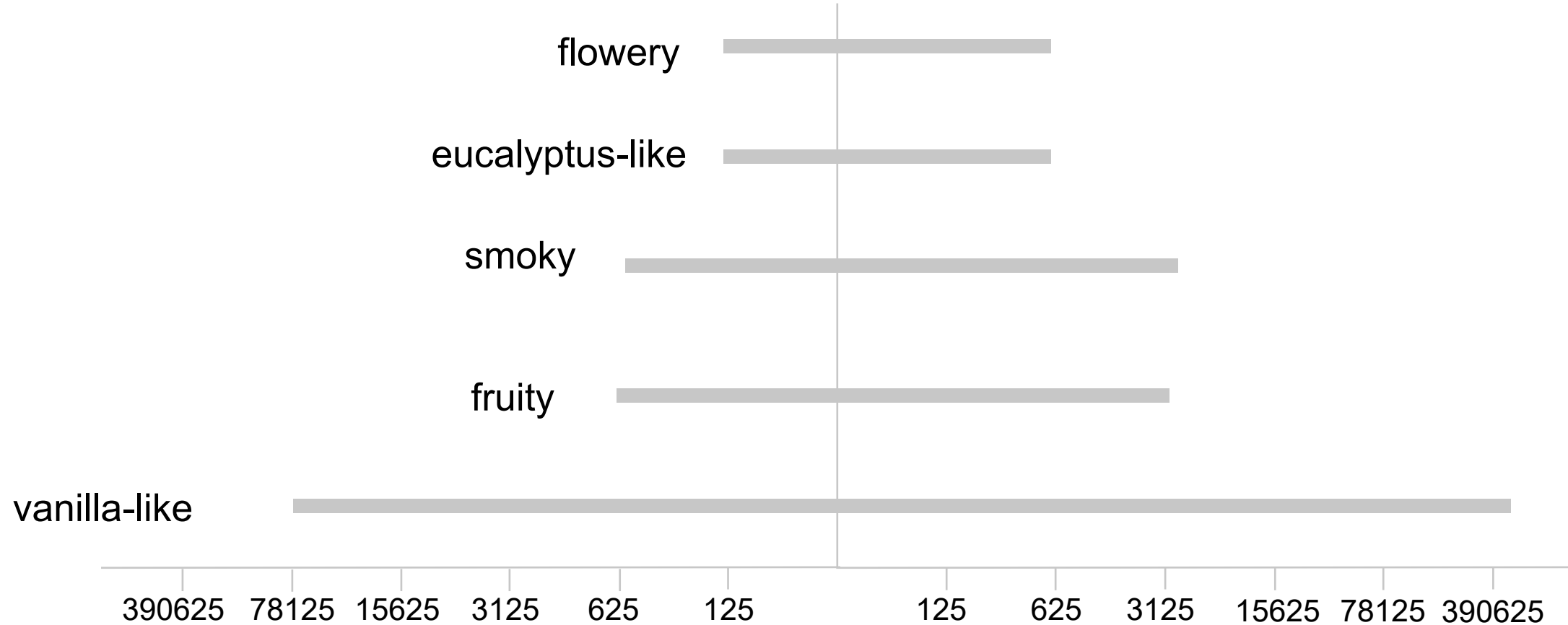
Voting for the attributes + finding the references (sniffing sticks)

Evaluation of overall intensity and hedonic rating

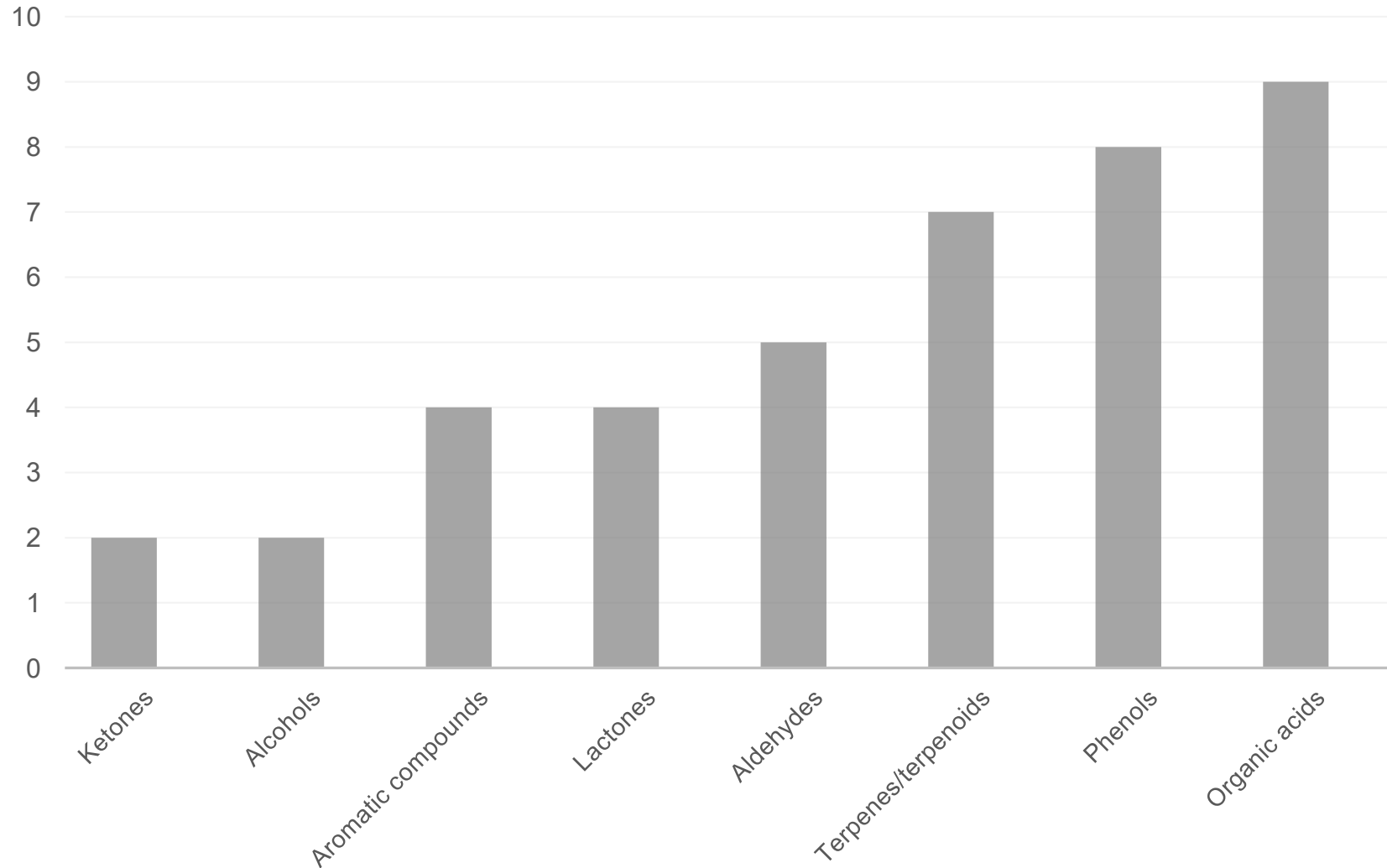
Flavor Dilution (FD) factors in the same sample across different years

2023

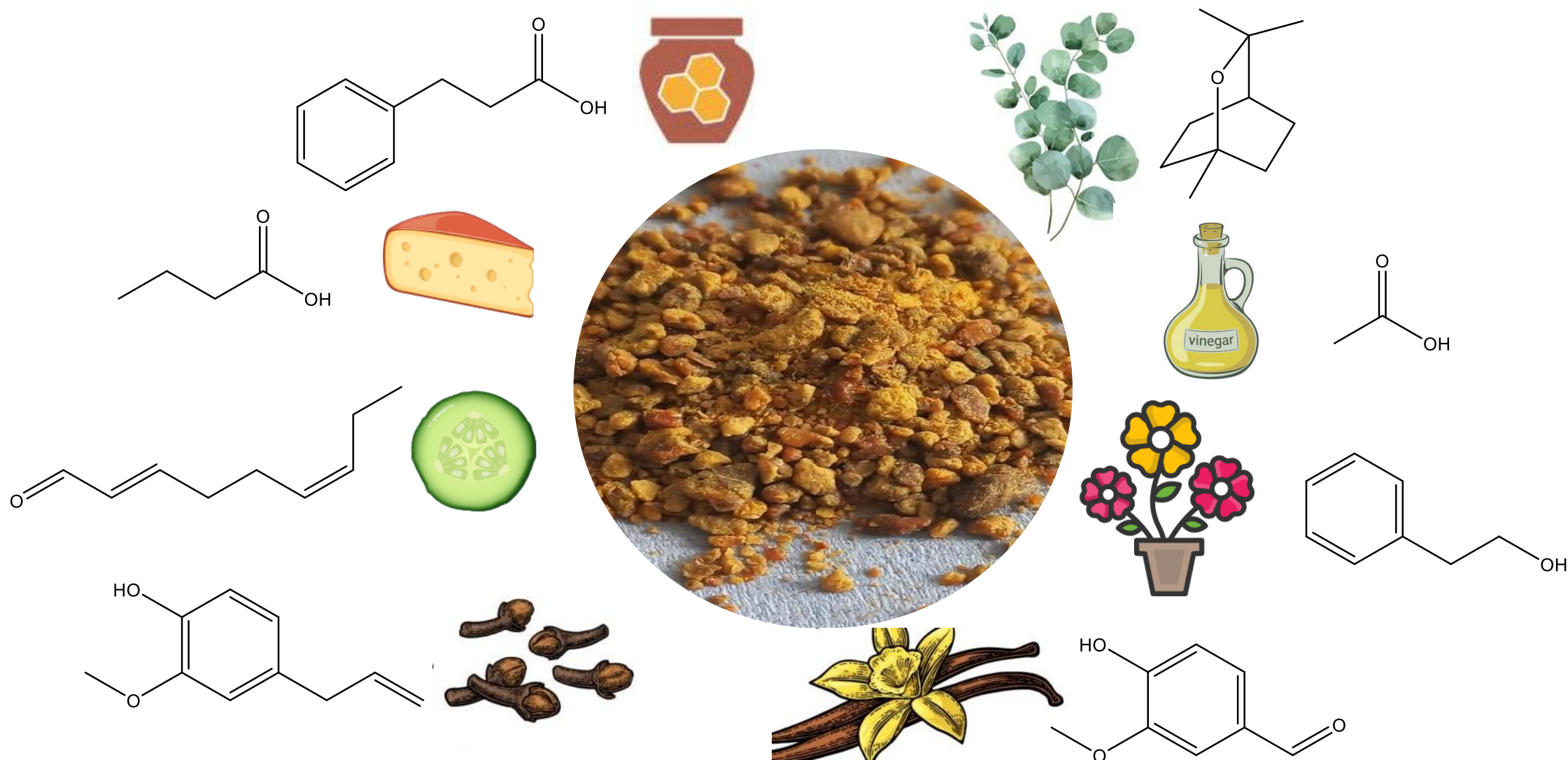
2024



Odor-active compounds according to the chemical group

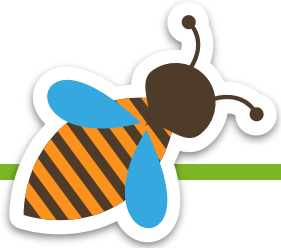


Identified odorants



- First study on aroma-active compounds in propolis from different climate regions within Europe
- Provides a foundation for future research on:
 - geographical variability of propolis composition
 - influence of different botanical sources
 - pharmacological properties of aroma-active compounds
 - antimicrobial potential in food preservation and safety
- The results contribute to developing quality control based on aroma profiles





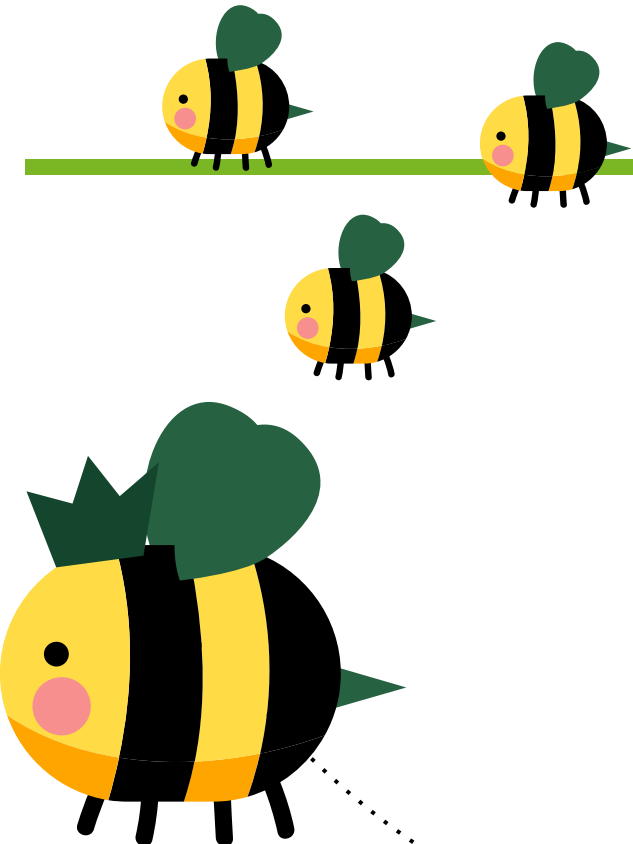
Acknowledgements

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Thank you for your attention!



astrid.sarapa@fau.de