

ANALYSIS OF AMINO ACIDS AND METABOLITES WITH GC-MS FOR THE DETERMINATION OF MONOFLORAL HONEYS



Tobias Wiezorek¹, Gudrun Beckh¹, Cord Lüllmann¹
Karl Speer²

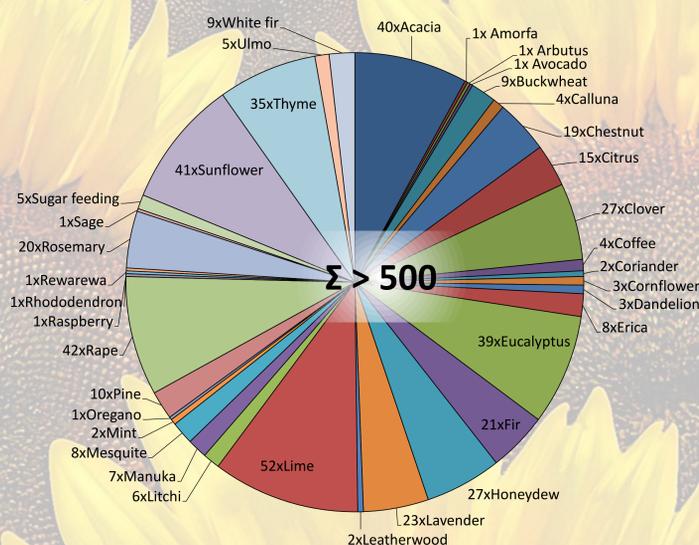


¹ Quality Services International GmbH
Flughafendamm 9a, D-28199 Bremen, Germany
info@qsi-q3.de

² Department of Food Chemistry
Bergstrasse 66, D-01062 Dresden, Germany
Karl.Speer@chemie.tu-dresden.de

The origin of monofloral honey has so far been determined by melissopalynology^[1]. This method, however, requires extensive experience and is time-consuming. Honey on average contains 980 mg/kg of amino acids^[2]. The individual concentrations are quite different but proline mostly dominates. By analyzing the amino acid spectrum, a new attempt for an alternative/standardized method to determine the origin of honey should be found.

The basis of this study should be a large number of different monofloral honeys, so that possible marker substances could be significantly funded and a multivariate data analysis can be made. More than 500 monofloral honeys were collected worldwide (Fig. 1). Each sample was analyzed by melissopalynology and supplementary criteria^[2] for its floral origin.



A method based on derivatization with n-propylchloroformiate and measurement with GC-MS was developed^[3] and validated. Only 35 minutes (two minutes manual work) are required for sample preparation including derivatization and GC-MS analysis per sample.

In addition to amino acids their degradation products were observed in the chromatogram, which reacts as well as the amino acids with this special derivatization reagent. An overview of the tested analytes is shown in Tab. 1. The quantification of amino acids was carried out by isotopically labeled standards. The metabolites were initially quantified in the first estimate by comparing peak areas of standard vs. sample.

All substances were detected in the analysis of honeys. Searching for markers, results of all analytes were displayed by using boxplots. A number of substances seem to be characteristic or at least in combination with other parameters specific to individual monofloral honeys. Amino acids and metabolites from the shikimic acid and phenylpropanoid metabolism are of particular interest since these occur only in plant organisms and not in honey bees.

Anthranilic acid in large quantities is characteristic for chestnut honey (Fig. 2). Anthranilic acid is the precursor of tryptophan in the shikimic acid pathway, which is mainly found in eucalyptus und thyme honey. No correlation was observed between precursor and product, so that different plants seem to apply different metabolic depots.

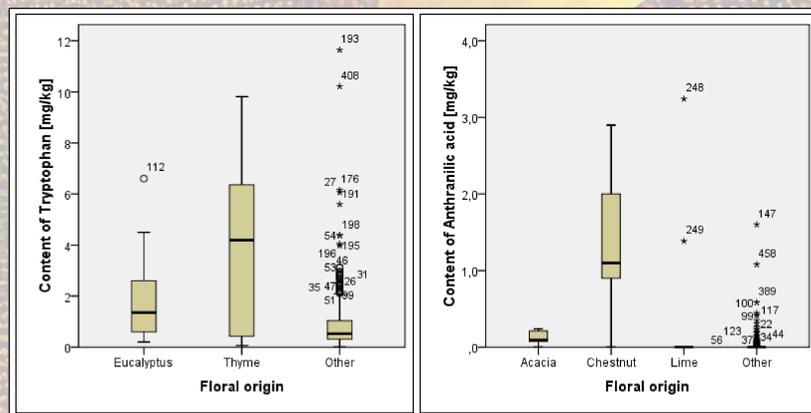


Fig. 2: Boxplots of amino acids and metabolites from shikimic acid pathway

Phenylalanine and tyrosine are products of the phenylpropanoid pathway and are both formed from prephenylic acid. These substances occur characteristically in lavender and thyme honeys (Fig. 3). Degradation products of these two substances are phenylacetic acid and trans-cinnamic acid, which occur characteristically in heather honeys, and only in small amounts in lavender and thyme honeys.

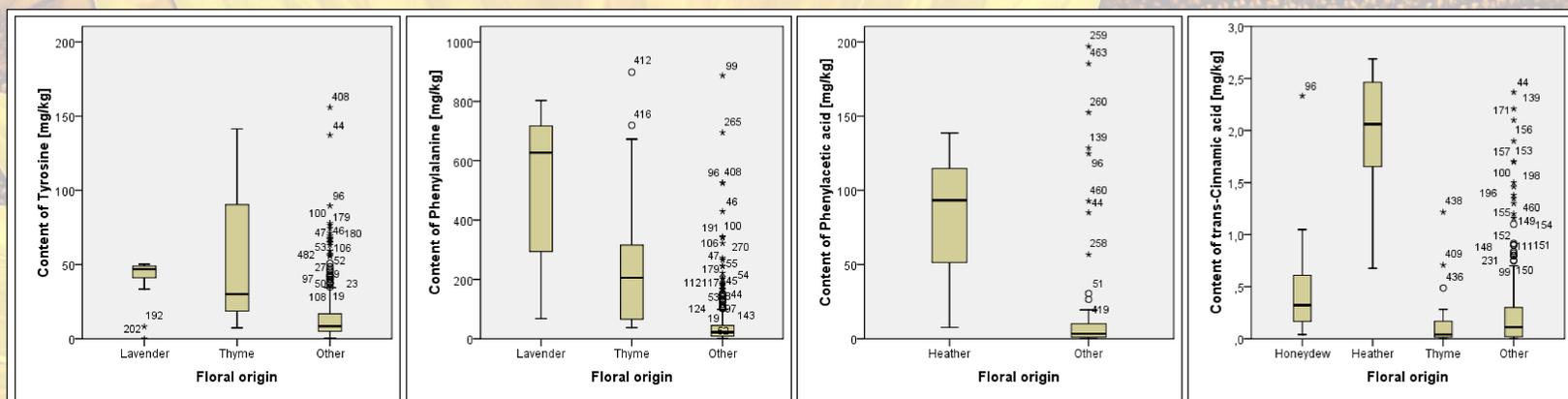


Fig. 3: Boxplots of amino acids and metabolites from phenylpropanoid metabolism

The objective is the determination of minimum levels of characteristic markers, so that limit criteria for certain monofloral honeys can be determined (see Guidelines for honey: content of methyl anthranilate in citrus honeys of at least 2 mg/kg). In addition, a statistical model should be developed, which enable the characterization of the floral origin of an unknown sample.

Analytes
Alanine
β-Alanine*
Asparagine
Aspartic acid
Glutamine
Glutamic acid
α-Aminobutyric acid*
β-Aminobutyric acid*
γ-Aminobutyric acid*
Glycine
Histidine
Leucine
Iso-Leucine
Lysine
Ornithine
Phenylalanine
Benzoic acid*
para-Coumaric acid*
Ferulic acid*
3,4-Dihydroxybenzoic acid*
3-Hydroxybenzoic acid*
4-Hydroxybenzoic acid*
Caffeic acid*
Mandelic acid*
Phenylacetic acid*
Phenyllactic acid*
Phenylpropionic acid*
Vanillic acid*
trans-Cinnamic acid*
Proline
Sarcosine
Tryptophan
Anthranilic acid*
Tyrosine
Valine

Tab. 1: Analyte (*=Metabolite)

Monofloral honeys contain characteristic amino acids (or metabolites) which allow determination of the botanical origin.

[1] Beckh, G., Camps, G., Deutsche Lebensmittelrundschau 105 (2009), p. 105-110.

[2] Lüllmann, C., Horn, H., Das große Honigbuch, 2. Aufl. (2002), p. 102, 106.

[3] Nozal, Ma.J., Bernal, J.L., Toribio, M.L., Diego, J.C., Ruiz, A., Journal of Chrom. A, 1047 (2004), p. 137-146