IDENTIFICATION OF MARKER PROTEINS FOR THE DETERMINATION OF MONOFLORAL HONEYS

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Aside of the pollen varieties, honey contains species of proteins at concentrations of 0.2 to 2%, mostly originating from honey bees, and to a lesser degree originating from floral sources[1]. By using various enzymatic detection reactions, specific plant proteins in honey can now be analyzed in order to develop an alternative/standardized method (beside melissopalynology[2]) to determine the origin of honey.

The basis of this study should be a large number of different monofloral honeys, so that possible marker proteins 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 melisso-palynology and supplementary criteria[2] for its floral origin.

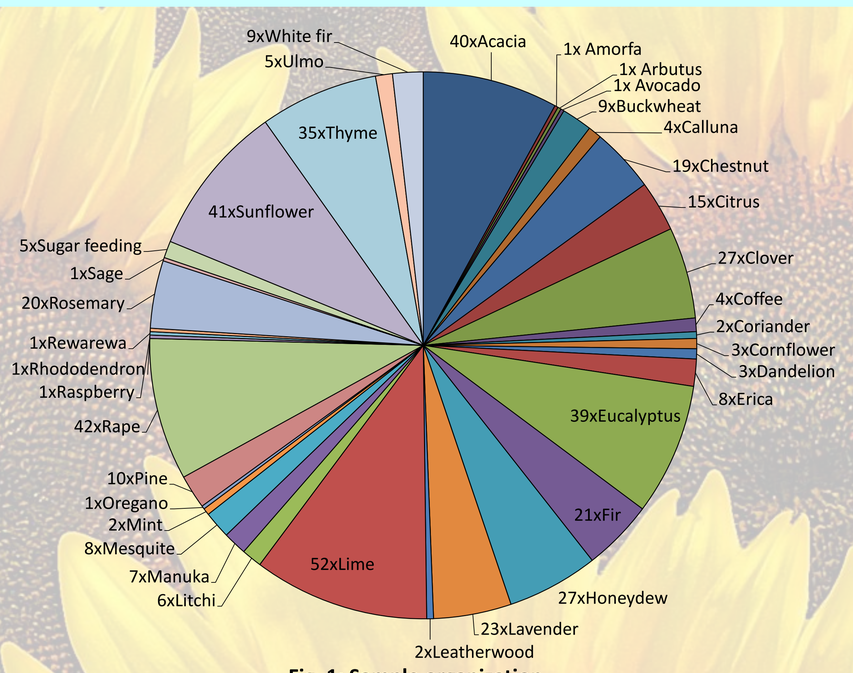

Fig. 1: Sample organization

Simple and rapid photometric methods were used or developed and validated for the analysis. Following parameters can be measured from one weighted sample:

- Amylase activity (DIN 10750 mod.)
- Invertase activity (DIN 10759/1 mod.)
- Acid phosphatase activity (measured via degradation of p-nitrophenylphosphate)
- Glucose oxidase activity (measured via the formation of hydrogen peroxide)
- Catalase activity (measured via degredation of hydrogen peroxide)
- Protein content (Bradford method[3])

To determine glucose oxidase in the presence of catalase and vice versa, a specific enzyme reaction were exploited and validated for the selective determination of each enzyme.

The measurements were performed by using a random access analyzer.

All analyzed enzymes were detected. A large variation of the activities is striking in descriptive statistics (**Tab. 1**). It can be assumed that proteins in honey have other sources besides the honey bee.

n = 507		Minimum	Median	Maximum
Amylase	DZ	n.n.	20	45
Invertase	U/kg	n.n.	74	284
Acid phosphatase	U/kg	n.n.	4,9	209
Catalase	U/kg	n.n.	1,6	25
Glucose oxidase	U/kg	n.n.	1	17
Protein	mg/kg	165	520	2050

Tab. 1: Descriptive statistics of measured data

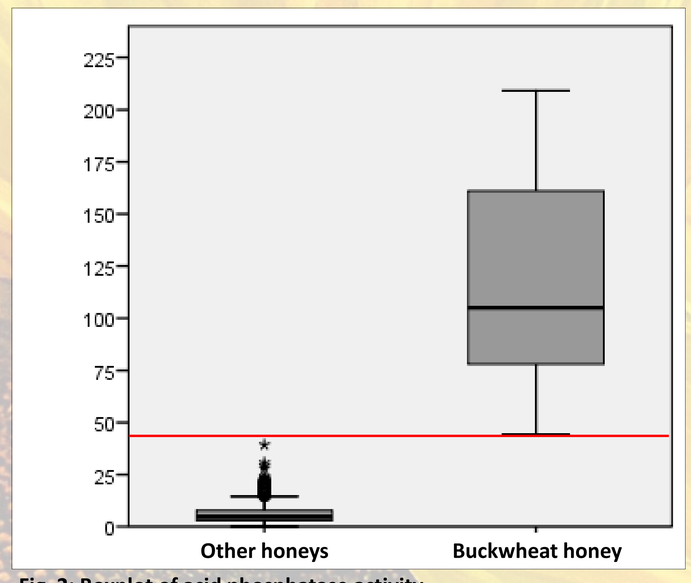

Fig. 2: Boxplot of acid phosphatase activity

Boxplot graphics were created for all parameters to reveal unique characteristics that show significant differences in honeys. Buckwheat honey clearly stands out for its higher acid phosphatase activity distinct from the population of other honeys **(Fig. 2)**. An acid phosphatase activity of >35 U/kg is typically for buckwheat honeys.

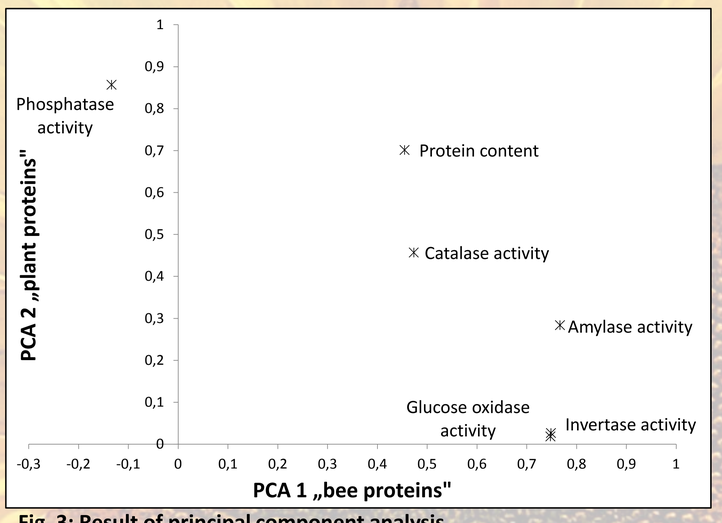

Fig. 3: Result of principal component analysis

Furthermore, the data were analyzed by principal component analysis and narrowed down to two principal components (PCA, **Fig. 3**), which describe 61% of the total variance of the samples. PCA 1 can be interpreted as honey bee proteins (glucose oxidase, invertase, diastase) and PCA 2 as plant proteins (phosphatase). Catalase and protein content can be described by both factors and not clearly assigned to honey bee or plant sources.

In **Figure 4a**, buckwheat honey drops again, and a distinct cluster can be formed. *Calluna* and *Erica* honeys can also be distinguished **(Fig. 4b)**, but pollen of both species occurred in most analyzed samples and therefore, the clusters slightly mix up.

Citrus and acacia honey also form unique clusters, but they can not be clearly distinguished from each other. In **Fig. 4c** (a diagram of all honeys), this leads to an disorganized overlay.

For further differentiation of monofloral honeys, other parameters have to be analyzed additionally, for example the amino acid profile.

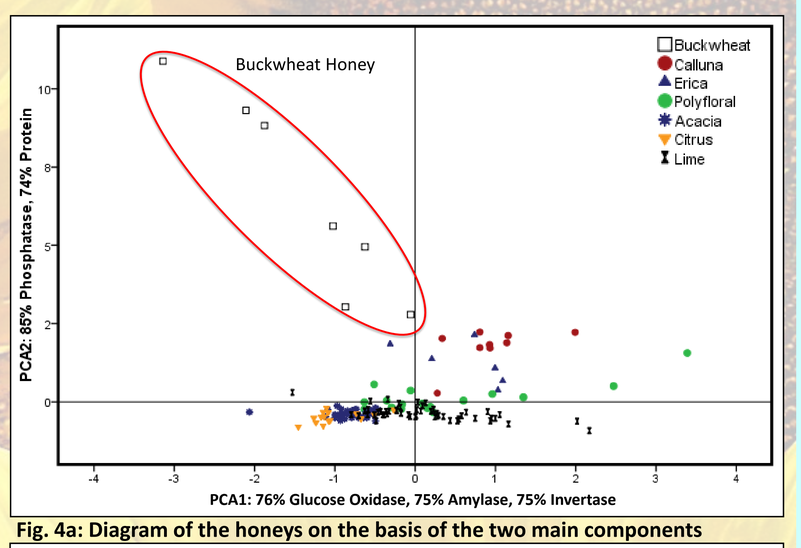

Fig. 4a: Diagram of the honeys on the basis of the two main components

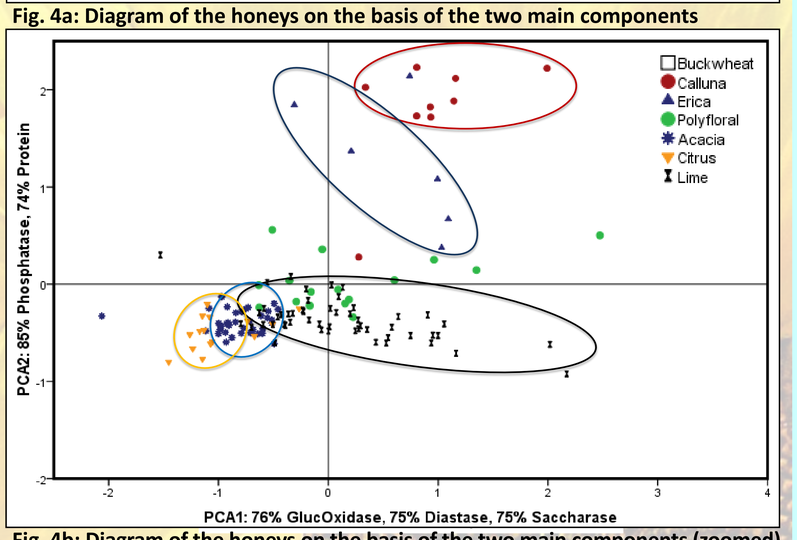

Fig. 4b: Diagram of the honeys on the basis of the two main components (zoomed)

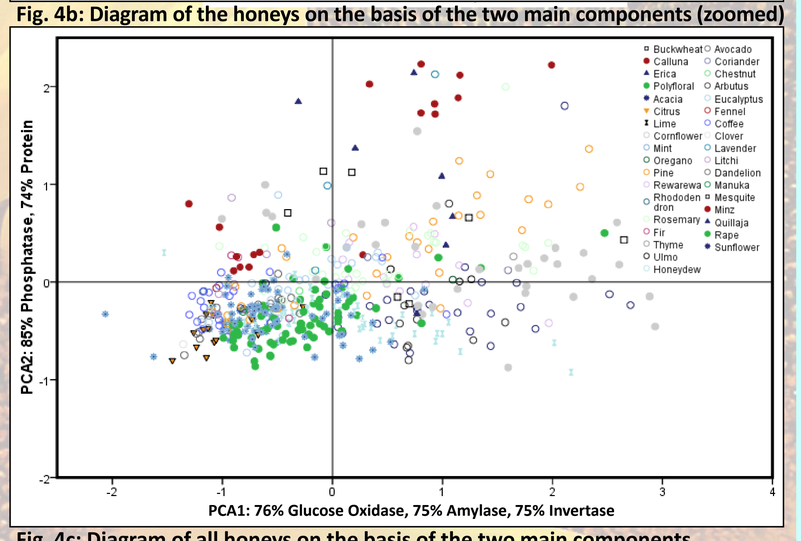

Fig. 4c: Diagram of all honeys on the basis of the two main components

Monofloral honeys contain marker proteins, which can be partly used to characterize the botanical origin.

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[1] *Lüllmann, C., Horn, H., Das große Honigbuch, 2. Aufl. (2002), p. 102*
[2] *Beckh, G., Camps, G., Deutsche Lebensmittelrundschau 105 (2009), p. 105-110*
[3] *Bradford, M.M., Analytical Biochemistry 72 (1972), p. 248-254*