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PP-189 [Beekeeping Technology and Quality]

Heather Honey Proteins

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Introduction

Honey derived from the nectar of heather (*Calluna vulgaris*) differs in many respects from most other floral honeys. Heather honey has distinct taste and flavor, as well as high commercial value. The protein content of heather honey can reach up to 2%, which is high compared to most honeys (usually < 0.5%). Mineral concentration is also high and can be up to 0,5 %. Its characteristic jelly-like consistency is thought to result from the presence of glycoproteins.

Nectar of heather presents a challenge for honeybees (*Apis mellifera*), and some bee strains are not able to produce ripe honey from it. Due to the high protein and mineral contents, heather honey can be unsuitable as winter food causing stress, dysentery, and even death of the colony, if the bees are not able to make cleansing flights early in the Spring. Heather honey can be identified organoleptically (taste, flavor, color, jelly-like consistency), as well as by measuring electric conductivity and detecting heather pollen in honey. In this study, we investigated the protein content of heather honey compared with other floral honeys, using samples provided by two beekeepers from Southwest Finland and commercially available products from Norway.

Results

The Finnish heather honey samples (samples 1-3) contained less heather pollen than the Norwegian samples (samples 7-9). Electric conductivities in the Finnish samples were also lower than in the Norwegian samples. Highest electric conductivities were measured in samples containing honey dew honey (samples 4 and 10), and these samples had low protein concentrations compared to heather honey. Highest protein concentrations were measured in Norwegian heather honey samples. We observed a strong correlation between heather pollen counts, protein concentration, and electric conductivity (with the exception of honey dew honey; **Fig. 1**)

Honey proteomes were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS; **Fig. 2**). The data were searched for bee and all green plant (*Viridiplantae*) proteins annotated in TrEMBL database. Majority of the soluble protein in the measured samples were secreted by bees. Despite the higher protein concentration, fewer proteins were identified in heather honey than in other floral honeys (**Fig. 3A**). The proteomes of the Finnish and Norwegian heather honeys exhibited similar features (**Fig. 3B**)

Sample	All nectariferous	Apiaceae	Brassicaceae	Calluna vulgaris	Cirsium/ Carduus	Impatiens	Myosotis	Pyrus / Malus / Aronia	Rosacea e	Salix	Taraxacum / Sonchus	Trifolium repens / hybridum	Trifolium pratense/ incarnatum / medium	Vaccinium	Others	Nectarless
1	358	41	91	40	1	25	1	2	65	10	1	26	x	25	30	69
2	406	13	14	55	1	26	4	x	167	25	1	48	1	20	31	8
3	422	12	9	13	15	15		33	99	177	x	19		11	19	7
6	423	17	2	x		x	300	2	37	24		24		9	8	19
8	362	2	x	205	4	1		1	42	27	8	7	3	1	61	65
9	367	2	57	134	2	x		1	79	15	2	32	10	2	31	63
10	403	1					300	1	64	12	3	5		14	3	14
11	422	4		1		x	350	1	26	19		x		12	9	4

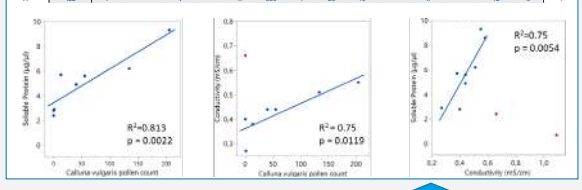

Figure 1. Pollen analysis. Top: Pollen counts by species or genus of plants identified in selected samples Bottom: correlations between protein concentration and heather pollen counts (left), electric conductivity and heather pollen counts (middle), and protein concentration and electric conductivity (right). Samples containing honey dew honey (red dots) were excluded from calculations involving electric conductivity

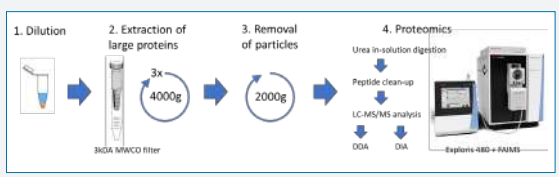

Figure 2. Proteomics workflow. Honey samples were diluted with water and proteins were extracted with 3 kDa molecular weight cut off centrifugal filter. Large particles, such as pollen, were then pelleted from the remaining sample, and soluble protein was prepared for LC-MS/MS analysis with a routine urea in-solution digestion method. The purified peptides were analysed with 2h gradients both with data-dependent acquisition method (DDA) for species identification and data-independent acquisition method (DIA) for quantitative comparisons.

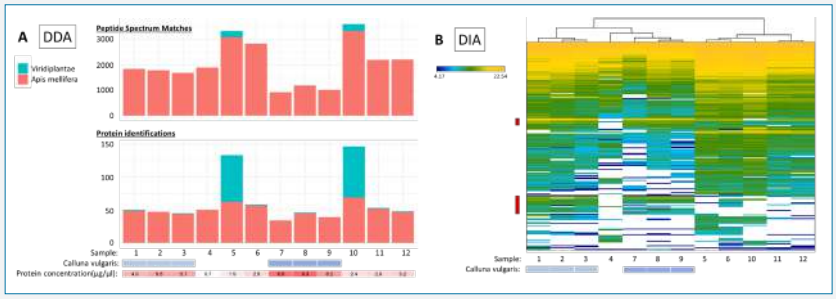

Figure 3. Honey proteome. A) Peptide spectrum matches (top) and protein identifications (bottom) for each sample in the DDA data searched against all sequences of the species *A.mellifera* and clade *Viridiplantae* in TrEMBL database. **B)** Hierarchical clustering of the samples based on DIA data shows similarities between Finnish and Norwegian heather honey samples. Hierarchical clustering of the proteins found two clusters that are to some degree specific to heather honey (indicated with red bars).

Table 1. Proteins predominantly found in heather honey. Proteins associated with DNA damage repair are shown with red color.

Uniprot ID	Protein Descriptions	Log2 Fold Change
A0A7M7G0B4	Transferrin	only in heather honey
A0A7M7GKH7	P-type Cu(+) transporter	only in heather honey
A0A7M7MNL5	Protein TAPT1 homolog isoform X2	only in heather honey
A0A7M7MQN6	Breast cancer type 2 susceptibility protein	only in heather honey
A0A7M7MBE7	Myotoperoxidase	only in heather honey
A0A7M7MTQ0	Hybrid signal transduction histidine kinase M	only in heather honey
A0A7M7R8M0	NAD-dependent protein deacetylase sirtuin-1 isoform X2	only in heather honey
A0A7M7G8P5	Uncharacterized serine-rich protein C215.13	10.24
A0A7M7GW34	Uncharacterized protein LOC102653840	9.04
A0A7M7GU48	Tetratricopeptide repeat protein 39 homolog, mitochondrial-like	7.50
A0A7M7GPF3	Uncharacterized protein LOC411955 isoform X2	5.87
A0A7M7GUP4	Disks large-associated protein 5 isoform X3	5.78
A0A7M7TFF5	Enhancer of mRNA-decapping protein 4	4.25
A0A7M7FZV4	Multiple inositol polyphosphate phosphatase 1	3.88
A0A7M7MK65	Dnal homolog subfamily C member 7 isoform X4	3.76
A0A7M7LKP7	Glycerate kinase-like	3.70
A0A7M7G9H6	DNA repair protein XRCC1	3.58
A0A7M7G8G4	Uncharacterized protein C9orf47 homolog	3.19
A0A7M7MTY1	Transcriptional regulator ATRX homolog isoform X3	2.96
A0A7M7LJF5	U6 snRNA-associated Sm-like protein LSm6	2.24

Discussion

The electric conductivity measurements were performed with a method described by Sancho et al. (1991). These values need to be multiplied by a factor of 1.5 to obtain values comparable with the standard method. The honey directive defines the upper limit of electric conductivity in floral honeys to 0.80 mS/cm. Two of the Norwegian heather honey samples (7 and 8) measured in this study had conductivities higher than limit in the honey directive. Given the high commercial value of heather honey, adjusting the limit set in the directive should be considered.

The soluble proteins in heather honey are mostly secretions of the hypopharyngeal glands, such as proteins belonging to the Major royal jelly protein family. However, the amount of protein secreted by bees is much higher in heather honey than in most other floral honeys, which may indicate that ripening of heather honey is more demanding for the bees. Some of the identified bee proteins exhibited a degree of specificity towards heather honey. As an interesting observation, several of these proteins have been associated with DNA damage repair.

References

1. Brother Adam: Breeding the honeybee. Northern Bee books. 1985
2. Kirkevold, Roar Ree. Gjaering i vestlandshonnigen. Birökteren 139(1) 8-9. 2023
3. von der Ohe, W. and von der Ohe, K. Ein ganz besonderer Honig. Deutscher Imker Journal 28 (8), 20-21. 2020
4. Sancho, M.T., Muniategui, S, Huidopro, J.F. And Simal, L. Correlation between the electrical cnductivity of honey in humid and dry matter. Apidologie 22, 221-227. 1991

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Ling heather (Calluna vulgaris) honey has many special characteristics: thixotrophy, strong flavor and taste, challenging to extract, high mineral content and high protein content. Ripening of heather honey is a heavy task for the bees and its water content can be quite high. Therefore, EU Food legislation allows heather honey to have,more moisture than other types of honey. It is known, that some bee strains are not able to produce ripe heather honey, and it has been suggested that this is linked to thickening of ripe honey. Our aim in this work was to study the proteins of heather honey, The samples included honey from two beekeepers in southwest Finland and commercially available Norwegian heather honey packed in packing enterprises. For comparison we had samples harvested prior to blooming of heather. We performed liquid chromatography mass spectrometry-based proteomics analysis of the soluble proteins and studied their species of origin. To estimate the amount of heather honey in the samples, we studied them organoleptically (taste, flavour, thixotrophy), by pollen analysis, and measuring electric conductivities. The samples contained both bee and plant proteins. However, when pollen and insoluble proteins were separated by centrifugation, the remaining soluble protein consisted predominantly of bee proteins, secreted from hypopharyngeal glands. The concentrations of bee proteins was higher in samples with higher fraction of heather honey, and we hypothesize that this contributes to the heavy burden of drying the heather nectar, linked to its composition and high humidity. Protein content, electric conductivity, and the amount of heather pollen were higher in Norwegian than Finnish amples, suggesting that they contained less of other types of honey, As the electric conductivity in samples with high fraction of heather honey can exceed 0,8 mS/cm limit set for blossom honeys in the honey directive, the need for adjusting the directive will be discussed.