A STUDY OF FREE AMINO ACID COMPOSITION OF DIFFERENT HONEYBEE PRODUCTS

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INTRODUCTION and AIM

Due to their nutritional, prophylactic, and therapeutic properties, honeybee (*Apis mellifera*) products (HBP) have been used since ancient times. They are complex mixtures that contain a huge variety of bioactive compounds, such as proteins, peptides, carbohydrates, minerals, biogenic amines, and other metabolites. However, the composition of HBP and their biological activity remains not fully investigated [1].

The study aimed to identify and quantify free amino acids occurring in three HBP: venom, pollen, and royal jelly. Moreover, an assessment of the variability of free amino acid profiles of HBP was also performed.

METHODOLOGY

HBP samples were collected from apiaries located in Poland. A validated method employing a liquid chromatograph coupled to a triple quadrupole mass spectrometer was used (Fig.1, Tab.1, Tab. 2). An amine-reactive isotope-coded tag (aTRAQ reagent, Sciex) was used for sample preparation.

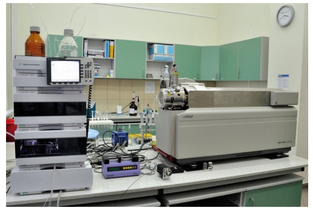

Fig. 1. A triple quadrupole 4000 QTRAP (Sciex) mass spectrometer coupled to a 1260 Infinity (Agilent Technologies) liquid chromatograph.

Tab. 1. LC parameters of the method used.

PARAMETER	SETTING
Chromatographic column	AAA C18, 4.6×150 mm (Sciex)
Mobile phase	Eluent A: 0.1% formic acid and 0.01% heptafluorobutyric acid in water Eluent B: 0.1% formic acid and 0.01% heptafluorobutyric acid in methanol
Flow rate	0.8 mL/min
Temperature	50 °C
Elution profile	gradient
Total run time	18 min
Injection volume	2 µL
Detector	Triple quadrupole mass spectrometer

Tab. 2. MS parameters of the method used.

PARAMETER	SETTING
Ionization method	Electrospray
Ionization mode	Positive
Scan mode	Multiple reaction monitoring
Temperature	600 °C
Ion spray voltage	4500 V
Curtain gas	20 psig
Declustering potential	30 V
Entrance potential	10 V
Collison gas	Nitrogen
Collision energy	30 eV (exceptions: cystine, homocystine, cystathionine, argininosuccinic acid, lysine hydroxylysine, and ornithine- 50 eV)

RESULTS and DISCUSSION

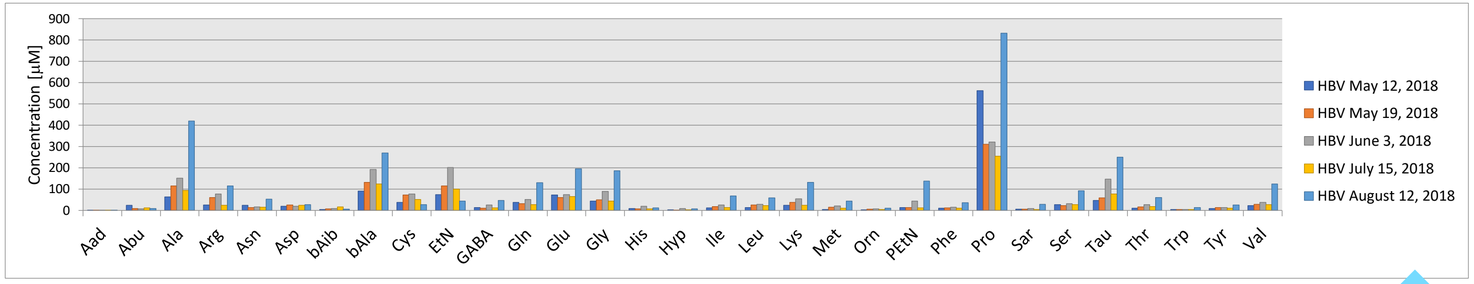

Fig. 2. Amino acid profiles determined in solutions of honeybee venom (c=50 mg/ml) collected in different months.

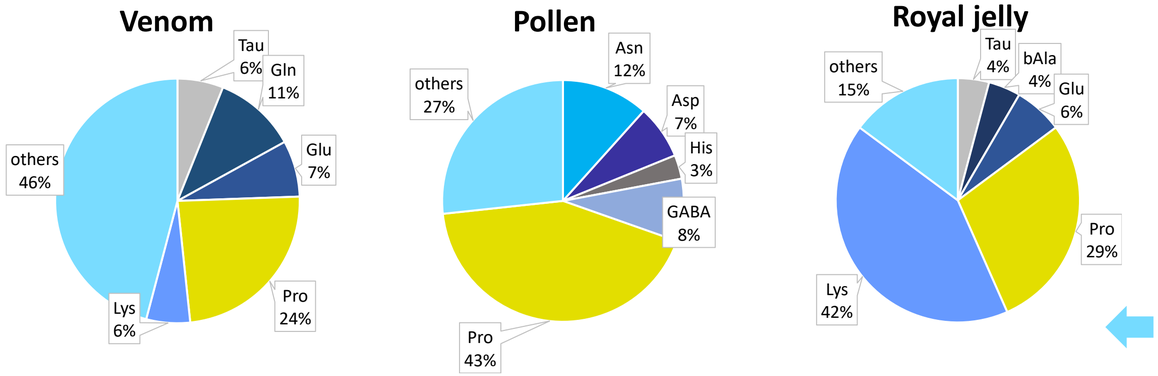

Fig. 3. A pie chart showing amino acids determined in the highest amount in honeybee venom.

- The study allowed us to obtain a broad amino acid profile of each HBP covering more than 30 compounds (proteinogenic and non-proteinogenic amino acids) (Fig. 2).
- The differences in amino acid profiles between bee venom samples collected within the same year, but in various months indicates a chemical variability of HBP, which poses a limiting factor in the use of HBP in medicine and dietary supplements (Fig. 2). Therefore, strategies for HBP standardization should be established.
- In honeybee venom and pollen, proline was the most abundant amino acid. In royal jelly, lysine and proline were the dominant constituents of the determined metabolite profile (Fig. 3). Proline is a major component of antimicrobial peptides occurring in HBP [2], which may explain the high level of this amino acid in the studied samples.

Acknowledgments
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References

[1] Klupczynska A, Pawlak M, Kokot ZJ, Matysiak J, Application of Metabolomic Tools for Studying Low Molecular-Weight Fraction of Animal Venoms and Poisons. *Toxins* 2018;10(8):E306.
[2] Wu Q, Patočka J, Kuča K, Insect Antimicrobial Peptides, a Mini Review. Toxins 2018;10(11):461.