

Characterization of Amino Acids, Polyphenols and Flavonoids of Some Bee Products Collected in the Banat Region

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Abstract

Bee products have a well-known tradition in human apitherapy, due to their numerous healing properties. The aim of this study was to evaluate the chemical characterization of the amino acid, polyphenol and flavonoid content of some bee products, such as apilarnil, royal jelly and propolis. The samples were collected from the apiary located in Caransebeș municipality, Banat region, Romania, in 2022, as well as from the market. Apilarnil and royal jelly were analyzed in pure and lyophilized form, and propolis in pure form and tincture. Our results show that there is great variability in the chemical composition of the three beekeeping products studied, depending on the form of presentation and the source of origin. In the case of amino acids, the highest values were obtained in royal jelly from the own apiary 39.93 mg/g, and for polyphenols and flavonoids, the best results were obtained in the case of pure propolis samples 40.82 mg GAE/g, respectively 268.11 mg QE/g.

Keywords: apilarnil; royal jelly; propolis; amino acids; polyphenols; flavonoids.

1. Introduction

Beekeeping, an ancient activity, represents an important pillar for human health and well-being due to the valuable bee products obtained from this practice. Honey, apilarnil, royal jelly and propolis are recognized as functional foods, due to their remarkable biotherapeutic properties, which include antioxidant, antimicrobial, anti-inflammatory and immunostimulatory effects [1, 2].

The Banat region of Romania has a remarkable honey biodiversity that provides a favorable

framework for beekeeping and the production of high-quality bee products [1,3,4]. The varied flora and favorable climatic conditions contribute to a significant chemical and biological diversity of these products. This diversity gives the region's beekeeping products a special nutritional and therapeutic value, attracting the interest of researchers in the fields of biochemistry and food sciences [5].

Amino acids, polyphenols and flavonoids are key bioactive compounds that play an essential role in the health benefits of these products.

Amino acids are involved in fundamental metabolic processes, polyphenols act as powerful antioxidants, and flavonoids contribute to cellular protection and immune system support. The variability of the concentrations of these

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compounds is influenced by several factors, including the type of beekeeping product, the processing form (pure, lyophilized or tincture) and the region of origin [3,6].

Our study explores the characterization of these substances in apilarnil, royal jelly and propolis, emphasizing the variability determined by the processing form (pure, lyophilized and tincture) and the source of origin. In addition, differences related to the form of processing and source of origin are explored, thus contributing to a better understanding of the therapeutic and nutritional potential of these beekeeping products. This detailed analysis provides valuable insight into the importance of beekeeping products from Banat in the context of public health, the food industry and from a biotechnological potential perspective.

2. Materials and methods

2.1. Samples analyzed

Some of the apilarnil and royal jelly samples were collected from the company's own apiary in Caransebeș, Banat region, Romania, in 2022, and the rest came from the market. Propolis was evaluated in pure form from the own apiary and from the market, as well as tincture, using standard extraction methods prepared by macerating 20 g of propolis (from the own apiary) in 100 ml of alcohol (Table 1). These were stored in glass containers at a temperature of $0\pm 5^{\circ}\text{C}$ in a refrigerator. The analyses were performed at the Multidisciplinary

Research Unit (PCI) of the University of Life Sciences "King Mihai I" from Timișoara.

2.2. Methodology of analysis

Amino acids: Determined by high-performance liquid chromatography (HPLC), analyzing the profile of essential and non-essential amino acids. The method allowed for the precise determination of compositional variations depending on the source and form of processing [7]. To determine total amino acids, 1 g/sample was weighed into each plastic container, to which 24 ml of 70% alcohol was added, thus resulting in a dilution of 1:250 for all beekeeping products. These were homogenized with the DLAB shaker (SK-L330-PRO, China) for 30 minutes, and subsequently filtered with filter paper and pipetted 1 ml of the resulting extract into a glass tube for each determination. 0.5 ml of Phosphate buffer solution (pH – 8.04) 1/15 mol/L + 0.5 ml Ninhydrin (2% + 0.8 mg/ml $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) was added to the test tube, then they were stirred using the DLAB stirrer (SK-L330-PRO, China) for 30 minutes, and were subsequently placed in an oven (BINDER GmbH, Tuttingen, Germany), at a temperature of $103\pm 2^{\circ}\text{C}$, for 10 minutes. After removing the test tubes from the oven, they were each filled with distilled water up to 10 ml and homogenized manually. Finally, they were left to rest for 15 minutes, after which the absorbance was read at 570 nm using a UV-VIS spectrometer (Analytical Jena Specord 205, Jena, Germany). All determinations were performed in triplicate.

Table 1. Bee product samples analyzed

No. sample	Product type	Source of origin	Abbreviation
1	Propolis raw	own apiary	PS
2	Propolis raw	Market	PC
3	Propolis tincture	Own apiary	PS
4	Royal jelly pure	own apiary	LMS
5	Lyophilized royal jelly	Market	LML
6	Royal jelly pure	Market	LMC
7	Apilarnil pure	own apiary	AS
8	Lyophilized apilarnil	Market	ALC
9	Apilarnil pure	Market	AP

Polyphenols: The determination of the total content was carried out using the Folin-Ciocalteu method, expressing the results in mg gallic acid equivalents (GAE) per gram of beekeeping

product. From the beekeeping products: "LMS"; "LML"; "LMC" and "AP", 1 g/sample was weighed into a plastic container with a lid, to which 10 ml of 70% alcohol was added, thus

resulting in a dilution of 1:10. Also from the bee products: "PS"; "PC"; "PT"; "AS" and "ALC", we used the dilutions of 1:250 in order to be able to take readings on the UV-VIS spectrometer (Analytical Jena Specord 205, Jena, Germany). The samples were homogenized with the DLAB shaker (SK-L330-PRO, China) for 30 minutes, and then filtered with filter paper and pipetted 0.5 ml of each filtered sample into a glass tube. Then we applied the titration process with 1.25 ml Folin-Ciocalteu reagent (Sigma-Aldrich Chemic GmbH, Munich, Germany), diluted 1:10 with distilled water and placed in each of the previously prepared test tubes. The prepared samples were incubated for 5 minutes at room temperature, and 1 ml of Na₂CO₃ (60g/L aqueous solution) was added to each and subsequently placed in an incubator (Mettler GmbH, Schwabach, Germany) at 50°C for 30 minutes. After this period, the absorbance was read at 750 nm with a UV-VIS spectrometer (Analytical Jena Specord 205, Jena, Germany), using the ethanol solution as a control, and the device calibration curve was obtained using gallic acid (concentration range: 2.5-250 µg/mL) [8,9]. All determinations were performed in triplicate.

Flavonoids: The analysis was based on the AlCl₃ method, with results expressed in mg quercetin equivalents (QE) per gram [10]. For the determination of flavonoids, we prepared containers with lids, in which the following dilutions were made: 1:10 of the beekeeping products "LMS"; "LML"; "LMC" and "AP", where 1 g/sample was weighed in a plastic container with a lid, over which 10 ml of 70% alcohol was added, and for the beekeeping products "PS"; "PC"; "TP"; "AS" and "ALC" dilutions of 1:250 were used, respectively at 1 g/sample and 24 ml of 70% alcohol were added, in order to be able to take readings on the UV-VIS spectrometer (Analytical Jena Specord 205, Jena, Germany). These were homogenized with the DLAB shaker (SK-L330-PRO, China) for 30 minutes, and then filtered with filter paper and 1 ml of the resulting extract was pipetted together with: 4 ml H₂O, 0.3 ml NaNO₂5%, 0.3 ml

Al(NO₃)₃10% and 2 ml NaOH1M in glass test tubes, after which they were homogenized and completed with 70% alcohol up to 10 ml, and finally the samples were left for 15 minutes at rest. Absorbance was read at 510 nm using UV-VIS spectrometer (Analytical Jena Specord 205, Jena, Germany). Quercetin solution was used as a control sample, and the results were expressed in mg QE/100g. All determinations were performed in triplicate.

The results obtained were statistically analyzed using Minitab 21 Statistical Software.

3. Results and discussion

1. Amino acids

The results obtained by spectrophotometry highlight a variable distribution of amino acids between the three beekeeping products analyzed: apilarnil, royal jelly and propolis.

Pure apilarnil (AP) from the market had the highest average concentration of amino acids (127.51 mg/g), which can be explained by its processing process and controlled provenance. In contrast, the apilarnil from the own apiary (AS) had lower values (40.82 mg/g), indicating a possible influence of environmental or processing factors on the content (Figure 1).

Pure royal jelly from the own apiary (LMS) recorded high concentrations (39.93 mg/g), confirming its nutritional potential and its essential role in feeding queen bees [1,2,11].

However, freeze-dried royal jelly (LML) showed a significant reduction in amino acid content, suggesting losses caused by the freeze-drying process [12,13].

Propolis did not show detectable concentrations of amino acids, confirming the fundamental differences in chemical composition between this product and the other two.

This variability highlights the importance of processing methods on preserving nutritional qualities. Minimal processing is ideal for preserving essential amino acids, which are critical for metabolic, immune, and cellular functions of the human and animal body.

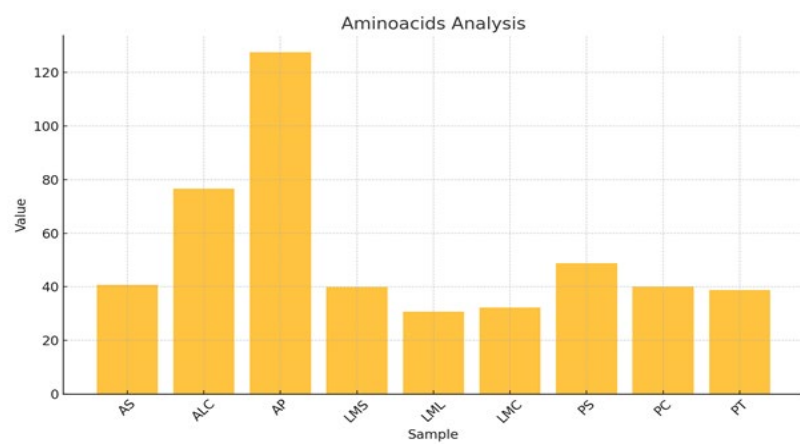


Figure 1 - Distribution of amino acid concentrations in the three analyzed bee products

2. Polyphenols

Determinations carried out using the Folin-Ciocalteu method have shown that propolis is the richest source of polyphenols. Pure propolis (PS) from the own apiary had an average concentration of 40.82 mg GAE/g, significantly higher than propolis from the market (32.16 mg GAE/g). This highlights the importance of the source of origin and the authenticity of the product [14]. Propolis tincture (PT) recorded reduced values compared to pure propolis, suggesting that the alcoholic extraction process can significantly

affect the concentration of polyphenols. Polyphenol levels in royal jelly were low (<1.07 mg GAE/g), indicating that this product does not represent a major source of phenolic compounds (Figure 2).

Polyphenols are known for their powerful antioxidant properties, and the high concentrations in propolis highlight its use in apitherapy and nutrition [2,9,15,16,17]. Differences between sources of origin underscore the need for standardization and certification of beekeeping products to guarantee quality [18,19].

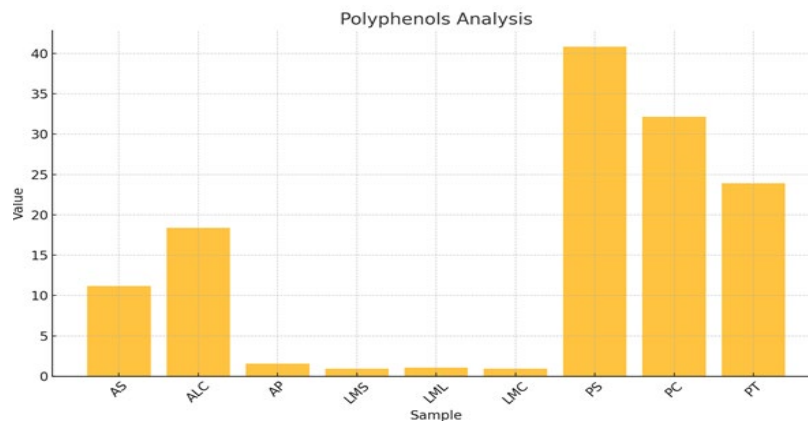


Figure 2 - Distribution of polyphenol concentrations in the three analyzed beekeeping products

3. Flavonoids

The AlCl₃ method demonstrated that propolis is the richest source of flavonoids among the products analyzed. Propolis tincture (PT) had the lowest concentration of flavonoids (72.21 mg

QE/g), which indicates that alcoholic extraction influences the release of these bioactive compounds (Figure 3).

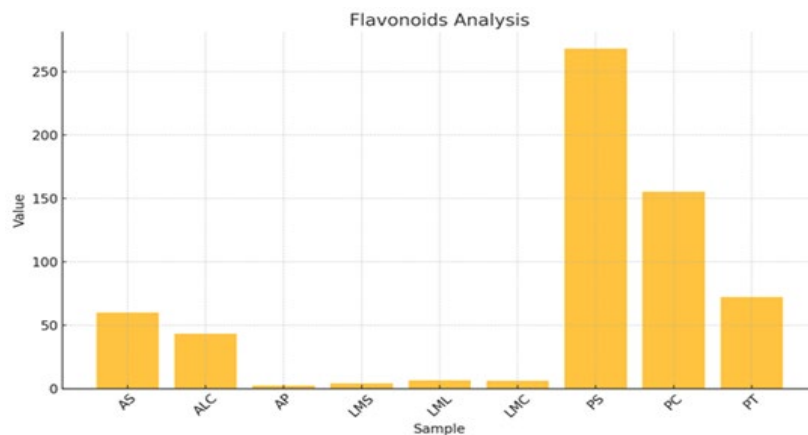


Figure 3 - Distribution of flavonoid concentrations in the three analyzed bee products

Pure propolis from the own apiary (PS) had the highest flavonoid values (268.11 mg QE/g), consolidating the position of propolis as a chemically superior beekeeping product.

Apilarnil recorded significantly lower values, and royal jelly values ≤ 6.33 mg QE/g, indicating secondary roles of these products in providing flavonoids.

Flavonoids in propolis are particularly valuable due to their anti-inflammatory, antimicrobial [1,2,20] and cardioprotective effects. Our results confirm that pure propolis, but also in tincture form, due to the high concentration of flavonoids, are suitable for therapeutic applications [21].

4. Conclusions

Source quality: Propolis, apilarnil and royal jelly from the own apiary demonstrated a superior composition compared to products on the market, emphasizing the importance of their source of origin.

Impact of processing: Minimal processing, such as using pure products, preserved high concentrations of bioactive compounds (amino acids and polyphenols). In contrast, methods such as freeze-drying can lead to significant losses in terms of chemical composition.

Therapeutic potential: Propolis, due to its high concentration of polyphenols and flavonoids, is particularly valuable for medical and nutritional uses. Propolis tinctures, in particular, represent an optimal form of administration for maximizing the bioavailability of active compounds.

Product specificity: Each beekeeping product has unique characteristics that determine its nutritional and therapeutic value. While apilarnil and royal jelly are excellent sources of amino acids, propolis stands out for its high content of polyphenols and flavonoids.

Recommendations:

Development of quality standards for beekeeping products that include chemical composition analysis [22];

Increasing use of propolis and tinctures in dietary supplements and pharmaceuticals due to their complex chemical composition;

Promoting minimally invasive processing methods for preserving bioactive compounds in all beekeeping products [23].

These conclusions provide a solid framework for future research on the use of bee products in nutrition and medicine.

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