EXPLORING THE COMPOSITION OF PROPOLIS AS A SUBJECT OF PROCESSING INTO FOOD PRODUCTS

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The object of the research is samples of propolis collected by various means from different regions of Ukraine. The main problem that is being solved is the search for optimal, efficient, and food industry-approved methods of collecting propolis as a raw material of processing into food products. The influence of the main methods of propolis collection on the key quality indicators of propolis for its application as a raw material in the food industry has been studied. The differences in the main raw material indicators across regions of Ukraine have been evaluated. The use of propolis collection methods that do not meet the safety requirements of the food industry is a common practice in beekeeping farms. The acceptability of the raw material for use in the food industry is based on its compliance with the requirements of current regulatory and legal acts on quality. However, the updating and revision of regulatory acts in view of production realities occur slowly and with significant delays. This approach reduces the volume of raw materials available for industrial use due to technical barriers and outdated regulatory acts on quality. In the course of the research, results were obtained based on such indicators as the mass fraction of wax, mechanical impurities and flavonoid compounds in propolis collected from three regions of Ukraine. The levels of indicators in the studied samples do not meet the requirements defined by DSTU 4662:2006. At the same time, the regulatory requirements of DSTU 4662:2006 and the research methods do not align with the finalized project ISO/DIS 24381, which is currently in the final stages of adoption as the primary international standard. The use of means of collection in the production of propolis raw materials, which are allowed to come into contact with food products, taking into account also the review of quality regulatory acts and bringing them into line with international documents, can contribute to improving the availability of this product as a food raw material. Propolis producers should pay attention to the sources of propolis located in ecologically clean areas with minimal industrial impact and adhere to proper beekeeping practices to obtain high-quality raw materials. The obtained results can be used to develop an industrial technology for the production of propolis as a raw material for food production.

Keywords: propolis, collection method, wax fraction, mechanical impurities, identification, flavonoids.

1. Introduction

Propolis is a sticky, resinous substance collected by bees from the buds, leaves, and stems of wild plants and processed, which has bactericidal properties and is used for sealing cracks in the hive, polishing the walls of wax cells, embalming the corpses of stung enemies (mice, reptiles, etc.) [1]. Propolis is used in the food industry as an ingredient in food products, edible coatings, and intelligent packaging. Considering the specific organoleptic characteristics of propolis, its application in the food industry involves deep processing. To achieve this, propolis is extracted with ethanol, water, subjected to lyophilic, vacuum, freeze-drying, electrostatic precipitation, and biotransformation [2]. Propolis prevents lipid oxidation and improves the shelf life of food products, including vegetables, fruits, and beverages. It can also be used as a safe, new, and natural preservative for meat and fish. The antibacterial and antioxidant properties of propolis are mainly attributed to compounds present in its composition, such as polyphenols and flavonoids [3]. The chemical composition of propolis generally includes resinous substances, accounting for about 50 %, beeswax 30 %, aromatic compounds and oils 10 %, pollen and mechanical impurities 5 % [4]. Propolis from different geographical regions contains over 800 different chemical components and compounds [5].

Depending on the botanical source, geographical origin, chemical composition, propolis is divided into the following types: Aspen Type Propolis, Mediterranean Propolis, Poplar Type Propolis, Pacific Propolis Type, Brazilian Green Propolis, Brazilian Red Propolis, Mangifera Propolis Type [6].

The chemical composition and biological properties (antibacterial, antifungal, antiviral, antioxidant and cytoprotective activity) of propolis extracts collected from different regions of Poland were investigated [7]. The total content of phenols (116.16–219.41 mg GAE/g EEP) and flavonoids (29.63–106.07 mg QE/g EEP) in propolis extracts depended on their geographical origin. The high content...
of epicatechin, catechin, pinobanksin, myricetin and vanillin and syringic acids in propolis samples was confirmed by chromatographic analysis. The values of total flavonoid content in propolis extracts collected from different regions of Poland range from 29.63 to 106.07 mg quercetin equivalent (QE)/g EEP. The highest total flavonoid content was found for propolis from Western Pomerania (106.07 mg QE/g EEP) and propolis from Greater Poland (Polonia Maior) (101.22 mg QE/g EEP). The lowest total flavonoid content (TFC) was determined for propolis from the Lublin Voivodeship (30.41 mg QE/g EEP), propolis from Mazovia (31.70 mg QE/g EEP) and propolis collected from the Subcarpathian Voivodeship (29.63 mg QE/h EEP). Flavonoids are often identified in propolis, but their profile and content vary depending on the geographical origin of the propolis. The content of flavonoids in the investigated propolis extracts indicates that P. nigra may be one of the plant sources used by bees for its production. Thus, P. nigra buds contain various flavonoids, including pinocembrin, pinobanksin, chrysin, galangin, vanillin, apigenin, and pinostrobin, which were also detected in some tested propolis extracts. The lowest total concentration of all analyzed flavonoids was determined for propolis from Lower Silesia (3.118 mg/g EEP), propolis from Lublin Voivodeship (3.200 mg/g EEP).

The poplar type of propolis contains a typical chemical profile, including a high level of flavonanes, flavones, and a low content of phenolic acids and their esters. Propolis from Romania, as a poplar-type product, has the main plant origin of resin from Populus species, but according to the geographical regions of collection, may have secondary resin sources such as Quercus, Aesculus, Ulmus, Picea, Salix and Frazinus. Different studies show that Romanian propolis extracts contain flavonoids on average 250–300 mg/g of polyphenolic compounds with great variability depending on the geographical region (relief form), time of collection (month), method of collection (scraping from frames or propolis collection tools) and the last, but no less important extraction method. In total, five samples of propolis from Romania were studied and it was found that the total phenolic content is 123.922–155.279 mg/g of dry extract, and the total flavonoid content is 7.228–25.089 mg/g of dry extract [8]. In [9], the relationship between the size of the propolis fraction extracted with water and ethanol and the content of polyphenols, flavonoids and antioxidant capacity was investigated. Propolis was crushed into fractions containing small (d<600 µm), medium (600 µm<d<1.25 mm) and large (d>1.25 mm) propolis particles. It was established that the different content of flavonoids for the ethanol extract was 3.09 mg/g for the fine fraction, the average was 4.01 and 4.2 for the medium and large, respectively. Therefore, the extraction kinetic constant was 0.049, 0.320 and 1.105 for the respective fractions, respectively.

The content of flavonoids in the extracts of Populas balsamifera L, Populas nigra L and propolis from Lithuania was studied [10]. The total amount of flavonoids, mg RE/g dry weight, was 46.13, 24.76 and 18.79, respectively.

Other authors investigated propolis collected in Romania from industrial and agricultural areas [11]. A high content of heavy metals was found in the industrial zone with the following results, mg/kg: Cd = 0.080±0.006, Cu = 3.203±0.052, Zn = 4.195±0.067, Cr = 2.34±0.074, Pb = 0.651±0.063, Zn = 1.146±0.06, Mn = 2.18±0.067. Low concentrations of heavy metals were found in propolis from agricultural areas. In the industrial region, the concentration of cadmium was significantly higher in propolis compared to honey. The highest content of pesticides was also found in propolis from the industrial zone. Thus, the total concentration of dichlorodiphenyltrichloroethane (DDT) (0.0867 mg/kg) exceeded the maximum permissible level for food products by 1.7 times.

Another work related to the study of propolis from the Netherlands for the content of beeswax, which is collected by cleaning the elements of the beehive [12]. The wax content is set in the range from 1 to 42.5%. Other results indicate that the average beeswax content in propolis was 11.1%. In [13], 40 samples of propolis obtained from 7 regions of Turkey were studied. According to their results, the average value of wax obtained by petroleum ether extraction in propolis is 26.03±5.16 g/100 g, ranging from 13.83 to 37.58 g/100 g. Previous studies have shown that wax is almost 30% of propolis by weight of propolis. The authors of the work [14], based on the results of the study of Brazilian propolis, hypothesized that since plants produce an epicuticular wax that covers all above-ground parts, the beeswax contained in propolis may come from plant secretions. But several differences between the composition of beeswax and plant wax can be noted. For example, the latter contains alkenes, oleic acid, as important hydrocarbon components, and esters, which may predominate in plant waxes.

Propolis from Turkey (n=48) and from Serbia (n=12) was studied [15]. Pollen grains of 32 colonies and 75 taxa were identified in propolis from Turkey. Scientists note that pollen grains stick to propolis in the process of collection from plants and during storage in the hive. In other studies, it is noted that Anatolian propolis (Turkey) comes from many plant sources and contains pollen grains of Pinaceae, Quercus spp., Castanea sativa, Helianthus annuus, Asteraceae, Apiaceae, Cistaceae, Campanula spp., Fabaceae, Salix spp., Brassicaceae, Platanus spp., Centaurea spp., Populus spp., Tilia spp., Onobrychis spp., Juniperus spp., Acer spp., Anthemis spp., Poaceae [13].

A diverse pollen spectrum of nectar-bearing plants and plants that do not secrete nectar (Quercus spp., Pinus spp., Campanula spp., Campanulaceae, Cistaceae, Poaceae) was determined [16]. An increased percentage of glycerol was observed in most of the studied propolis samples. The reason for this is assumed to be the use of oxalic acid (with glycerol) for the treatment of bees against varroatozisis.

Plant fragments were identified in propolis from Brazil [17]:

- biseriate glandular trichome;
- epithelial cell;
- glandular trichome;
- leaf fragment;
- mesophyll;
- phloem;
- stalk cells;
- trichome;
- tector trichome;
- uniseriate glandular trichome.

During the study of the composition of Polish propolis, secretory discs and other plant particles, besides pollen grains, were also identified. It was established [18] that plant particles were identical to those isolated from fresh leaves of Betula spp. and Alnus spp. The presence of secretory discs in propolis samples supported the hypothesis that bees collected resinous substances from the surfaces.
of plants such as *Betula* spp. and *Alnus* spp. In [19], the presence of leaf primordia of *Zuccagnia punctata* (with a length of 440.23±167.87 µm), glandular trichomes (with a diameter of 163.75±93.12 µm), non-glandular trichomes (with lengths of 299.87±10.73 µm and 45.21±4.21 µm), leaf epidermal cells, and multicellular uniseriate non-glandular trichomes (with a length of 174.91±49.12 µm) was identified in the composition of propolis. Remains of pollen, spores, insect integuments, bristles, and hairs were observed in all propolis samples.

Other authors noted that in Poland, propolis is divided into two quality classes, which are determined based on the presence of substances and compounds insoluble in 95 % ethanol, tested after filtering the ethanol extract of propolis through filter paper [20]. Such substances include: wax, wood chips, dead bees and other solid contaminants. Propolis of the highest class can contain up to 30 % and of the lower class up to 50 % of insoluble substances.

Therefore, propolis in its composition contains wax, both of plant origin and added by bees during the accumulation of propolis in the nest, particles of the plant source of propolis, which are later identified as mechanical impurities. Compliance of propolis with the regulatory requirements of DSTU 4662:2006 and the international standard ISO/DIS 24381 is a condition for its further sale on the market as a subject of processing into food products.

**Aim of research** is to compare the quality of propolis obtained by various methods for its further use as an object of processing into food products.

### 2. Materials and Methods

The object of the study is propolis samples obtained by different means of collection in different regions of Ukraine. The work was carried out as part of the dissertation research on the topic «Scientific and technical support of the propolis production process and equipment» at the Department of Standardization and Certification of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine during 2020–2023.

To achieve the set goal, the following tasks were defined:
- organize the collection of propolis from apiaries in different regions of Ukraine, using collection tools with various technical characteristics;
- determine whether the size of the collection tool openings affects the wax content in propolis samples;
- clean the obtained propolis samples according to new and classical techniques;
- investigate the propolis samples for indicators such as wax content and mechanical impurities;
- determine the mass fraction of flavonoid compounds.

To obtain propolis samples using the new technology, 3 bee colonies were involved, and 3 grids (TM Stanz Pres, according to the manufacturer’s declaration, compliant with Commission Regulation (EU) No. 10/2011 of January 14, 2011) measuring 20x39 cm were placed. To obtain samples using classical techniques, the following methods were employed:
- 3 bee colonies were used and placed in 3 plastic grids measuring 180x245 mm (Lviv region);
- 6 samples were obtained using mosquito nets measuring 495x410 mm (Poltava and Ternopil regions).

The openings of the grids were measured using the TM Konus Crystal 7x–45x stereomicroscope (Italy) and the TM Sigeta MCMOS 5100, 5.1 Mp video camera (Ukraine) with ToupTek ToupView software (version 4.11.19728.20211022).

The TM Stanz Pres grids, plastic propolis collection grids, and mosquito nets were cooled at +5 °C for 60 minutes before cleaning. The TM Stanz Pres grids were cleaned using device [21], while the plastic propolis collection grids (beehive chisel) and mosquito nets were cleaned using the classic method.

The analysis of propolis samples was conducted at the Laboratory of Quality and Safety Assessment Methods for Beekeeping Products, National Scientific Center «Institute of beekeeping named after P. I. Prokopovich» (Kyiv, Ukraine), using standard methods (DSTU 4662:2006). The laboratory operates under the Certificate of Recognition of Measurement Capabilities No. PT-285/22 dated 12.12.2022.

The mass fraction of wax, mechanical impurities and flavonoids of propolis samples was studied according to the standard methods of DSTU 4662:2006, and statistically processed using Microsoft Excel tools.

### 3. Results and Discussion

#### 3.1. Results

Field researches were conducted, and propolis samples were collected in different regions of Ukraine (Lviv, Poltava, and Ternopil regions). The collection of propolis was carried out using three different tools: elastic mesh, plastic grid for propolis collection, and mosquito net.

Honeybees transport wax and propolis within the nest from one location to another. Both wax and propolis are used for scaling openings and gaps, in addition to constructing honeycombs [22, 23]. It is known that honeybees deposit propolis in openings with diameters ranging from 0.1 to 2.3 mm, while wax is typically deposited in openings ranging from 3.5 to 10 mm. To properly assess the impact of the opening sizes in the used tools, measurements of the openings were conducted (Table 1).

![Table 1](image)

Subsequently, the content of wax, mechanical impurities, and biological activity based on the flavonoid content were further investigated. According to the obtained results, the highest wax content in propolis was found when using mosquito nets in the Ternopil region (Table 2).

Among the investigated propolis samples, the highest wax content (49.57 %) was found in the sample obtained using a mosquito net from the Ternopil region, which is 3.24 % and 0.6 % higher compared to the samples obtained from Stanz Pres nets and plastic grids, respectively.
The highest content of mechanical impurities was found in the propolis collected using plastic grids in the Lviv region (39.83 %). The mass fraction of mechanical impurities in the propolis samples collected using plastic grids is 14.23 % higher compared to Stanz Pres nets and 6.96 % and 8.73 % higher compared to mosquito nets, respectively.

Mechanical impurities are a component of propolis, which, on the one hand, confirms its authenticity, and on the other, affects the compliance of the product with the requirements of regulatory documents on quality and admissibility of further use in the food industry. Microscopic examination of mechanical impurities contained on filter paper confirms the presence of plant residues, pollen and other non-intensified objects in propolis samples (Fig. 1).

![Image](building material)

These mechanical impurities confirm the authenticity of propolis. At the same time, unidentified objects of mechanical impurities of propolis require further research. Identification of objects by sources of origin in the future will enable the development of ways to minimize them in the technology of producing propolis as a raw material.

Currently, it is not known which specific plant sources of propolis in the temperate climate zone contribute to the increase in the content of natural mechanical impurities in the obtained samples.

The biological value of propolis, as a raw material and component of food products, lies primarily in the presence and quantity of flavonoid compounds (Table 3).
According to the requirements of DSTU 4662:2006, the mass fraction of flavonoid compounds in the investigated propolis samples should be at least 25 %. The research results showed that the mass fraction of flavonoid compounds in propolis samples exceeded the specified minimum in the standard, ranging from 2.31 % to 39.39 %. ISO/DIS 24381 (Bee propolis – Specifications) defines the levels of phenolic compounds and specifically flavonoids for poplar-type propolis depending on the research methods. The Polish standard PN-R-78891 (Propolis – kit pszczeli) does not establish requirements for determining the mass fraction of flavonoid compounds. The level of flavonoid compounds in propolis is important as it provides properties such as antibacterial and antioxidant effects [3]. The total content of phenols in propolis extracts from Poland in the studies was 116.16–219.41 mg GAE/g EEP and flavonoids 29.63–106.07 mg QE/g EEP. The collection location of the raw material affects the overall content of phenols and flavonoids in propolis. As mentioned by the authors in [7], propolis collected in the same country but different locations differ in chemical composition and biological properties. The diverse combination of bioactive compounds in propolis is of great importance for the biological activity of its extracts. Therefore, in the development of regulatory acts that regulate the quality of propolis, its variability in chemical composition and collection location should be taken into account.

The results of our study are limited by the conditions of sample collection (specified in the methodology). The limits of applicability of the results lie in their replication to the collection means used in the study (TM Stanz Pres nets, plastic grids for propolis collection and mosquito nets).

Further research on the composition of phenolic compounds will be promising, as it will allow the use of propolis in the development of recipes for functional products. Additionally, future studies may focus on determining regional types of propolis in Ukraine and assessing their biological activity as a raw material for health-promoting food products.

### 4. Conclusions

Minimizing the opening area of propolis collection devices does not affect the reduction of the proportion of wax in the propolis. The highest wax content in propolis, collected using mosquito nets placed in apiaries in Ternopil region, was 49.57±3.08 %, while the lowest (45.63±5.08 %) was from Poltava region. The difference in opening size between the Stanz Pres nets and plastic grids is 14.80 %. The wax content in propolis obtained from plastic grids is 2.64 % higher compared to samples collected using Stanz Pres nets.

The opening area of the mosquito nets is 34.09 % smaller compared to the Stanz Pres nets, while the wax content, on the contrary, is higher. This raises doubts about the previous claims that the opening area of the propolis collection nets affects the increase in wax content.

Samples of propolis collected using TM Stanz Pres nets contain the least amount of mechanical impurities. The proportion of mechanical impurities in propolis obtained from one region was 14.23 % higher in samples collected with plastic grids compared to Stanz Pres nets obtained from the same region.

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Conflict of interest

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Data availability

The data will be provided upon a justified request.

References


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