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# Roman Dvykaliuk

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

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 This approach reduces the volume of raw materials available for industrial use due to technical barriers and outdated regulatory acts on quality.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.

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# 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 vanillic 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 (34.70 mg QE/g EEP) and propolis collected from the Subcarpathian Voivodeship (29.63 mg QE/h EEP). Flavonoids are often identified in poplar-type propolis, but their profile and content vary depending on the geographical origin of the propolis. The flavonoid profile of 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 flavanones, 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 Fraxinus. Different studies show that Romanian propolis extract contains 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 7.728-25.089 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 *Populus balsamifera* L., *Populus 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 - 2.344±0.074, Pb - 0.651±0.063, Zn - 1.146±0.06, Mn - 2.184±0.067. Low concentrations of heavy metals were found in propo-

lis 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 52 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 varroatosis.

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 <code>Betula</code> spp. and <code>Alnus</code> spp. In [19], the presence of leaf primordia of <code>Zuccagnia punctata</code> (with a length of 440.23±167.87  $\mu m$ ), glandular trichomes (with a diameter of 163.75±93.12  $\mu m$ ), non-glandular trichomes (with lengths of 299.87±10.73  $\mu m$  and 45.21±4.21  $\mu m$ ), leaf epidermal cells, and multicellular uniseriate non-glandular trichomes (with a length of 174.91±49.12  $\mu m$ ) 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 180×245 mm (Lviv region);
  - 6 samples were obtained using mosquito nets measuring 495×410 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 sealing 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**The area of the openings of the propolis collection tools used in the study

Collection tool (n=10)	The area of the holes of propolis collection devices, mm <sup>2</sup>
Grids (TM Stanz Pres), $X\pm\Delta$	4.40±0.07
<i>Cv,</i> %	5.23
Plastic propolis collection grid, $X\pm\Delta$	29.72±1.76
<i>Cv,</i> %	18.73
Mosquito net (building material), $X\pm\Delta$	1.50±0.04
<i>Cv,</i> %	9.26

**Notes:** Cv – coefficient of variation, %; X – mean;  $\Delta$  – standard deviation

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.

Table 2
Summarized results of the study of propolis samples collected using various means

Method of collection $(n=3)$	Indicator	Mass fraction of wax, %	Mass fraction of me- chanical impurities, %
Grids (TM Stanz Pres)	<i>Cv,</i> %	34.86	28.99
	X±Δ	46.33±5.11	25.60±2.35
Plastic propolis collection grid	<i>ℂv,</i> %	12.40	8.01
	X±Δ	48.97 ± 1.92	39.83±1.01
Mosquito net (building material)	<i>ℂv,</i> %	19.66	18.11
	X±Δ	49.57±3.08	31.10±1.78
Mosquito net (building material)	<i>Cv,</i> %	35.19	20.59
	X±Δ	45.63±5.08	32.87 ± 2.14

**Notes:** Cv – coefficient of variation, %; X – mean;  $\Delta$  – standard deviation

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).



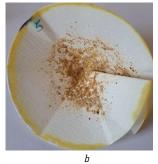


Fig. 1. Image of mechanical impurities obtained as a result of the study of propolis samples: a — image of mechanical impurities and plant residues under a microscope; b — image of filter paper with mechanical impurities of propolis

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).

Table

Mass fraction of flavonoid compounds in propolis samples collected using different means

Method of collection $(n=3)$	Indicator	Mass fraction of flavonoid compounds, %
Grids (TM Stanz Pres)	<i>Cv,</i> %	23.91
	$X\pm\Delta$	31.66±2.39
Plastic propolis collection grid	Сv, %	41.12
	$X\pm\Delta$	27.31±3.55
Mosquito net (building material)	<i>Cv,</i> %	10.68
	X±Δ	64.39±2.18
Mosquito net (building material)	Сv, %	24.88
	$X\pm\Delta$	33.89 ± 2.67

**Notes:** Cv – coefficient of variation, %; X – mean;  $\Delta$  – standard deviation

The highest content of flavonoid compounds (64.39 %) was found in propolis collected from mosquito nets in the Ternopil region, which is 32.73 % more than in the samples collected in the Lviv region (TM Stanz Pres nets) and 37.08 % more, compared to the samples collected in the Lviv region (plastic grid). Along with this, the use of mosquito nets is not possible to obtain raw materials for the food industry. The obtained results can be used for further planning of the use of Ternopil region to obtain raw materials with a high content of flavonoids.

**3.2. Discussion.** According to Commission Regulation (EU) No. 10/2011 of January 14, 2011 on plastic materials and articles intended to come into contact with food, they should not transfer their components to food in quantities exceeding 10 milligrams of total released substances per square decimeter of the food contact surface (mg/dm²). Currently, beekeepers use mosquito nets for collecting propolis, even though the material is intended for the production of consumer goods and household use. Plastic grids, which are made from various types of plastics, do not indicate compliance with food contact requirements by either manufacturers or sellers. Only the manufacturer of Stanz Pres brand nets (Germany) declares compliance with food contact requirements.

According to the requirements of DSTU 4662:2006, the wax content in propolis samples should not exceed 15.0 %. In the examined samples, the wax content in propolis exceeded the requirements of DSTU 4662:2006 and ranged from 30.63 % to 34.57 %. The Polish standard PN-R-78891 (Propolis – kit pszczeli) sets requirements for substances insoluble in 95 % ethyl alcohol and classifies propolis into two categories, where samples with a content of up to 30 % belong to class 1, and samples with a content of up to 50 % belong to class 2. The project ISO/DIS 24381 (Bee propolis -Specifications) states that propolis may contain beeswax as an impurity and does not regulate the wax content in propolis or its permissible percentage. According to the results of the propolis research [12], the wax content was found to range from 1 % to 42.5 %. Other findings indicate average beeswax content in propolis of 11.1 %. In [13], 40 samples of propolis obtained from 7 regions of Turkey were studied. According to their results, the average wax content obtained through petroleum ether extraction in

propolis is  $26.03\pm5.16$  g/100 g, ranging from 13.83 % to 37.58 %. Despite wax being considered an impurity in propolis according to quality standards, several scientific studies have explored its application in the food industry. In [24], the possibility of using propolis wax as a substitute for palm oil in functional chocolate paste was investigated. In [25] it was noted that from the point of view of safety for human health, beeswax is considered a food product with the number E901, and propolis wax is a food ingredient generally recognized as safe (GRAS) [26]. Propolis wax is also used in the production of oleogels, which attracts manufacturers due to its ease of manufacturing, excellent composition of fatty acids, and safe usage in food products to meet consumer demands for healthy products [27]. Research [28] confirms the ability of wax, pollen, propolis, and bees to accumulate high levels of toxic and potentially toxic elements from the environment. Therefore, attention should be paid not only to the wax content in propolis but also to its quality.

According to the requirements of DSTU 4662:2006, the mass fraction of mechanical impurities in a propolis sample should not exceed 15 %. In the investigated propolis samples, the content of mechanical impurities exceeded the permissible level, ranging from 10.6 % to 24.83 %. The ISO/DIS 24381 (Bee propolis - Specifications) project states that propolis should comply with food legislation and standards regarding heavy metals, pesticides, toxins, pharmacologically active substances, and polycyclic aromatic hydrocarbons in each country. Therefore, ISO/DIS 24381 does not operate requirements for mechanical impurities and does not define methods for their determination. The Polish standard PN-R-78891 (Propolis - kit pszczeli) establishes requirements regarding impurities such as wax moth larvae at all stages of development, bitumen, putty, and other foreign odors, which serve as grounds for rejecting a batch of propolis. The determination of the content is recommended to be carried out using a magnifying glass with 10x magnification. The presence of pollen grains in propolis in significant quantities has been noted in [15]. In [19] the features of propolis with regard to the content of plant residues as a source of resin for the production of propolis, which they are, are proven. The issue of propolis adulteration is becoming more pronounced as the demand for natural food products grows. The presence of plant residues and pollen grains serves as markers of its authenticity and confirms its geographical origin [29, 30]. However, quality standards classify all insoluble substances and fragments in propolis as mechanical impurities, which deteriorate its quality.

The content of mechanical impurities is influenced by factors such as the presence of plant sources, sources of technological pollution, failure to adhere to proper beekeeping practices, and the use of inappropriate collection tools. Mechanical impurities should be classified into those of natural origin, technological origin, and man-made impurities. Such an approach, along with the appropriate research methods defined in quality regulatory documents, will enable manufacturers to adjust their content based on the results of the research, taking possible measures in relation to the source of origin. This, in turn, will contribute to improving the quality of the obtained raw material for use in the food industry.

Considering the negative impact of the conditions of martial law in Ukraine and ongoing environmental contamination, the investigation of the nature of mechanical impurities in food raw materials is particularly relevant and requires further research.

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) define 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  $49.57\pm3.08$  %, while the lowest  $(45.63\pm5.08$  %) 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**

The author declares that he has no conflict of interest in relation to the presented research results, including financial, personal, authorship or other nature, which could affect the research and its results presented in this article.

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## **Data** availability

The data will be provided upon a justified request.

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