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RESEARCH ARTICLE

Physicochemical Characteristics of Raw Propolis from Polish Urban Apiaries

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Abstract

Urban beekeeping is expanding, yet raw propolis from city apiaries remains poorly characterised. This study evaluated the physicochemical characteristics of raw propolis collected in 2019 from six Polish urban rooftop apiaries located in Kraków, Katowice, Warsaw, Legionowo, Łódź and Toruń. The samples were assessed macroscopically and analysed for wax, moisture, and 70% ethanol-soluble and insoluble fractions. They differed in colour and consistency, ranging from light-brown, loose material to dark-brown, compact and hard propolis. Wax and moisture contents ranged from 9.22 to 15.26% and from 5.66 to 10.34%, respectively, while the ethanol-soluble fraction ranged from 60.58 to 68.39%. Within this set of samples, the Kraków material had the lowest wax and moisture content and the highest soluble fraction, whereas the Łódź sample contained the largest insoluble fraction. These data demonstrate variability among the analysed raw propolis materials and identify the Kraków and Katowice samples as suitable candidates for further extraction studies. Because the study covered one apiary per city and a single collection season, broader multi-apiary and multi-year sampling is required before the observations can be generalised to individual cities or to urban propolis as a whole.

KEYWORDS

urban beekeeping; bee products; wax content; ethanol-soluble fraction; physicochemical characterisation

Introduction

Propolis, also known as bee glue, is a resinous material produced by honey bees *(Apis mellifera L.)* from plant exudates collected from buds, young shoots, bark fissures and other plant surfaces. The collected resin is transported to the colony and mixed with beeswax and bee-derived secretions. Within the hive, propolis is used to seal cracks, reduce the size of openings, reinforce structural elements and cover irregular surfaces. It also contributes to the colony's social immunity by limiting microbial growth on hive surfaces and by supporting the maintenance of a comparatively stable nest environment (Sforcin and Bankova 2011; Sforcin 2016).

Raw propolis is not a uniform material. It usually contains a resinous and balsamic fraction rich in plant secondary metabolites, together with variable quantities of wax, volatile compounds, pollen and minor organic and mineral constituents. The relative abundance of these fractions depends on the vegetation available within the foraging range, climatic and seasonal conditions, honey-bee behaviour, colony needs and harvesting practices. In temperate parts of Europe, propolis is commonly associated with Populus species and is often classified as poplar-type propolis; nevertheless, even materials belonging to the same broad chemical type may differ substantially in the concentrations and proportions of individual phenolic acids and flavonoids (Bankova 2005; Pobiega et al. 2023; Wagh 2013).

This natural variability is important from both analytical and technological perspectives. The colour of propolis may range from

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yellow-green to reddish-brown or almost black, whereas its consistency changes with temperature and composition from hard and brittle to plastic, sticky or highly adhesive. These properties influence grinding, homogenisation, solvent penetration, phase separation and filtration. A high wax content may reduce the proportion of extractable resinous constituents and complicate downstream processing, while excessive moisture may impair handling and storage stability. The proportions of ethanol-soluble and insoluble material therefore provide useful first-line information on the expected extraction yield and the amount of non-extractable material that will remain after processing (Dias et al. 2012; Feás et al. 2014; Grassi et al. 2023).

Most food, pharmaceutical and cosmetic applications use propolis extracts rather than the unprocessed material. Ethanol-water mixtures, particularly solutions containing approximately 70% ethanol, are frequently used because they recover a broad range of phenolic constituents while facilitating the removal of a considerable part of the wax and other insoluble components. However, extraction efficiency and extract composition depend not only on solvent concentration but also on the quality and homogeneity of the starting material. Consequently, macroscopic assessment and the determination of wax, moisture and extractable matter are relevant preliminary stages in propolis quality control, although they cannot replace chromatographic identification and the quantification of bioactive compounds (Papotti et al. 2012; Pavlovic et al. 2020; Pobiega et al. 2019).

Urban beekeeping has expanded in many European cities, increasing the availability and visibility of locally produced bee products. Urban landscapes may contain a heterogeneous mosaic of ornamental trees, parks, allotments, roadside vegetation and private gardens, potentially providing honey bees with diverse plant resources over an extended flowering period. At the same time, the composition and spatial distribution of these resources differ markedly between cities and neighbourhoods. Studies based on pollen metabarcoding have demonstrated that honey bees in complex urban environments may exploit a highly diverse flora, whereas the analyses of urban hive density have also emphasised that the ecological context of city beekeeping is location-specific (Casanelles-Abella and Moretti 2022; Fox et al. 2022). Such variation in resin-producing vegetation may be reflected in urban propolis, but raw propolis from city apiaries remains considerably less studied than urban honey.

A previous investigation of extracts prepared during the same 2019 sampling campaign demonstrated clear differences in phenolic composition and antioxidant activity. Despite this variation, the extracts showed a profile characteristic of European poplar-type propolis, with cinnamic acid derivatives among the dominant compounds (Pobiega et al. 2023). That publication focused on HPLC-DAD characterisation and the antioxidant activity of the extracts and did not report the raw-material parameters examined here: macroscopic appearance and consistency, wax content, moisture content, or the 70% ethanol-soluble and insoluble fractions. The present study therefore reports a complementary, physicochemical dataset for the raw propolis material.

Therefore, the aim of the present study was to compare the macroscopic and physicochemical characteristics of raw propolis collected from six Polish urban apiaries. Colour and consistency were evaluated together with wax content, moisture content and the proportions of 70% ethanol-soluble and insoluble matter. The study provides complementary raw-material quality data for previously characterised urban propolis extracts and identifies samples with the most favourable characteristics for further technological processing and evaluation as sources of bioactive compounds.

Materials and Methods

Raw propolis samples

Raw propolis was collected in 2019 from six urban rooftop apiaries in Poland: Kraków (50°03' N, 19°56' E), Katowice (50°15' N, 19°01' E), Warsaw (52°13' N, 21°00' E), Legionowo (52°24' N, 20°55' E), Łódź (51°45' N, 19°28' E) and Toruń (53°00' N, 18°35' E). At each apiary, propolis was collected on three separate occasions at approximately two-week intervals; depending on the location, the sampling series was conducted between May and August 2019. Each collection occasion constituted a separate batch. For each batch, propolis obtained from several hives available at that apiary was pooled to form one composite batch. The number of hives contributing to an individual batch varied according to hive availability. All apiaries contained more than five hives and were located on the roofs of one- to four-storey buildings in city centres. Propolis from Legionowo and Toruń was collected using propolis traps, whereas the remaining materials were scraped from frames and other hive elements. The samples were stored at -20°C at the Department of Food Biotechnology and Microbiology, Institute of Food Sciences, Warsaw University of Life Sciences (SGGW), until analysis. Before analysis, the frozen material was powdered. In the terminology used here, an apiary refers to the group of hives at one location, a hive to an individual colony, a batch to the material collected during one sampling occasion, a composite sample to material pooled from several available hives within that batch, and an analytical subsample to the portion withdrawn from an individual batch for a given assay. The three independently collected composite batches per apiary constituted the replicates used for a statistical analysis.

Macroscopic assessment

Each raw propolis sample was placed in a dry porcelain dish and assessed under natural daylight. Colour and consistency were described using visual and tactile categories such as light brown, brown or dark brown and loose, sticky, compact or hard. The assessment criteria were adapted from Kasote et al. (2017).

Determination of wax content

Powdered propolis (10.0 g) was placed in a glass bottle and mixed with 12.5 mL of deionised water. Wax was separated using the density-based microwave procedure described by Hogendoorn et al. (2013) and included among the standard methods for Apis mellifera propolis research (Bankova et al. 2019). The mixture was heated for 2 min in a microwave oven at 400 W until its temperature approached 100°C without prolonged boiling. After cooling to room temperature, the upper wax layer was transferred with a spatula onto a pre-weighed paper filter and weighed using an analytical balance (RADWAG, Radom, Poland). Wax content was calculated as the mass of separated wax divided by the initial sample mass and expressed as a percentage. The determination was performed separately for each of the three independently collected composite batches from each apiary.

Table 1. Macroscopic characteristics of raw propolis from Polish urban apiaries. Photographs show representative portions of the composite samples and are intended only for a qualitative comparison of colour and consistency. Image scale was not standardized during acquisition; therefore, a calibrated scale bar cannot be added retrospectively.

Apiary location	Photograph	Colour	Consistency
Kraków		light brown	loose
Katowice		dark brown	loose, sticky
Warsaw		dark brown	compact
Legionowo		brown	loose
Łódź		brown	loose, sticky
Toruń		light brown to dark brown	compact, hard

Determination of moisture content

Moisture content was determined using an oven-drying procedure modified from Feás et al. (2014). Powdered raw propolis (5.0 g) was spread on paper and dried in a convection oven (Zalmed, Poland) at 80°C for 2 h. The sample was cooled to room temperature and weighed using an analytical balance (RADWAG, Radom, Poland). The determination was performed separately for each of the three independently collected composite batches from each apiary. Moisture content was calculated as follows:

$$\text{Moisture content (\%)} = 100 \times (m1 - m2) / m1$$

where m1 is the mass of the raw propolis sample before drying (g), and m2 is the mass of the dried sample (g).

Determination of ethanol-soluble and insoluble fractions

The procedure was adapted from Feás et al. (2014). Powdered raw propolis (1.0 g) was mixed with 10 mL of 70% (v/v) ethanol and sonicated for 20 min in an ultrasonic bath (EMAG, Germany). The mixture was filtered through Whatman Grade 4 qualitative cellulose filter paper (Whatman/Cytiva; nominal particle retention 20-25 µm). The retained insoluble residue was dried at 80°C for 24 h, cooled to room temperature and weighed. The determination was performed separately for each of the three independently collected composite batches from each apiary. The insoluble fraction and the soluble fraction were calculated as follows:

$$\text{Insoluble fraction (\%)} = 100 \times mI / mS$$
$$\text{Soluble fraction (\%)} = 100 \times (mS - mI) / mS$$

where mS is the initial mass of raw propolis (g), and mI is the mass of the insoluble residue (g).

Statistical analysis

The results are presented as mean ± standard deviation (SD) of three independently collected composite batches per apiary. The three batches were collected on separate sampling occasions and were treated as independent batch-level replicates. Data were analysed using a one-way analysis of variance (ANOVA), with apiary/location as the factor, followed by Tukey's honestly significant difference test. Differences were considered significant at $p < 0.05$. Statistical calculations were performed using STATISTICA version 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

Results and Discussion

Macroscopic characteristics

The raw propolis samples differed visibly in colour and consistency (Table 1). The Kraków sample was light brown and loose, whereas the Katowice and Warsaw samples were dark brown. Propolis from Legionowo and Łódź was brown, while the Toruń material was heterogeneous and contained fragments ranging from light to dark brown. Consistency varied from loose to sticky, compact or hard. The Warsaw sample was compact, the Toruń sample was compact and particularly hard, and the materials from Katowice and Łódź were loose and sticky.

The observed diversity is consistent with the broad range of organoleptic properties reported for propolis from different geographical regions. Kasote et al. (2017) described Indian propolis samples as light brown, orange, green or brownish-brown and distinguished loose, compact, sticky and non-sticky materials. Grassi et al. (2023) likewise demonstrated significant colour variation among propolis samples collected within a relatively restricted geographical area in southern Italy. These findings indicate that substantial macroscopic variation can occur even when samples originate from one country or climatic zone.

Colour is generated by the combined contribution of plant resins, waxes, pollen, oxidised phenolic constituents and minor impurities. It may therefore provide useful descriptive information, but it cannot be used alone to determine botanical origin. This point is particularly relevant to the present material because the corresponding extracts were previously classified as European poplar-type propolis despite clear differences in the appearance of the raw samples (Pobiega et al. 2023). The light colour of the Kraków sample and the dark colour of the Katowice and Warsaw samples should thus be interpreted as phenotypic characteristics of the analysed materials rather than direct evidence of resin collection from specific plant species.

Consistency is influenced by temperature, the wax-to-resin ratio, particle size, storage history and the manner in which propolis is removed from the hive. At lower temperatures, wax and resin components become more rigid, whereas at higher temperatures the material becomes increasingly plastic and adhesive. The compact character of the Warsaw and Toruń samples was qualitatively consistent with their comparatively high wax contents of 15.26 and 14.19%, respectively. Nevertheless, the relationship was not absolute: the Toruń material was collected using a propolis trap, while the Warsaw sample was scraped from hive elements, and other compositional factors probably also contributed to texture.

Wax and moisture contents

Wax content differed significantly among the analysed apiaries and ranged from 9.22% in the Kraków propolis to 15.26% in the Warsaw propolis (Table 2). The value recorded for Warsaw was approximately 1.7 times that of the Kraków sample. The Toruń propolis also contained a relatively high proportion of wax (14.19%), whereas the Kraków and Legionowo propolis had the lowest values. According to Tukey's test, the Warsaw and Toruń propolis formed the same highest-value group, while the Kraków propolis showed the lowest mean value; the Legionowo propolis was intermediate and did not differ significantly from the Kraków propolis. These differences are technologically relevant because wax contributes relatively little to the phenolic fraction recovered during hydroalcoholic extraction and may increase the quantity of material that must be separated during filtration or cooling.

Table 2. Physicochemical characteristics of raw propolis from Polish urban apiaries.

Apiary location	Wax content (%)	Moisture content (%)	Insoluble fraction (%)	Soluble fraction (%)
Kraków	9.22 ± 0.48^{a}	5.66 ± 0.15^{a}	31.61 ± 2.34^{a}	68.39 ± 2.34^{a}
Katowice	12.12 ± 0.25^{b}	8.18 ± 0.10^{bc}	33.29 ± 2.98^{a}	66.71 ± 2.98^{a}
Warsaw	15.26 ± 1.17^{c}	10.34 ± 0.19^{e}	37.17 ± 5.34^{b}	62.83 ± 5.34^{b}
Legionowo	10.67 ± 0.93^{ab}	9.20 ± 0.17^{d}	36.98 ± 4.38^{b}	63.02 ± 4.38^{b}
Łódź	11.78 ± 0.78^{b}	8.29 ± 0.14^{c}	39.42 ± 5.55^{b}	60.58 ± 5.55^{b}
Toruń	14.19 ± 0.55^{c}	7.86 ± 0.24^{b}	37.88 ± 4.20^{b}	62.12 ± 4.20^{b}

Values are means ± SD of three independently collected composite batches per apiary. Different superscript letters within a column indicate significant differences according to Tukey's test ($p < 0.05$).

The wax levels obtained in the present study fall within the wide range described for the propolis of different origins. Negri et al. (1998) reported 2.3-16.4% wax in Brazilian propolis, while Feás et al. (2014) reported a mean value of 10.7% for Portuguese samples. In contrast, approximately 35% of wax was found in some Romanian propolis samples (Stan et al. 2011), and Grassi et al. (2023) reported an average of 27.98% in propolis from southern Italy. Thus, all six Polish urban samples were comparatively low in wax relative to the latter studies.

Harvesting practice may be one source of variation. Propolis traps are intended to yield relatively clean material, whereas scraping frames and hive surfaces can unintentionally introduce additional beeswax and fragments of hive material. Papotti et al. (2012) demonstrated that the method used to harvest Italian propolis influenced its chemical and functional characteristics. The high wax content of the scraped Warsaw propolis is compatible with a harvesting effect, but the similarly high value in the trap-collected Toruń propolis shows that collection method alone cannot explain the observed pattern.

Moisture content differed significantly among the analysed apiaries and ranged from 5.66% in the Kraków propolis to 10.34% in the Warsaw propolis (Table 2). The Kraków proplis had the lowest value and the Warsaw propolis the highest, while the Legionowo

proplis showed an intermediate but distinct value of 9.20%. The Katowice proplis (8.18%) did not differ significantly from either the Toruń propolis (7.86%) or Łódź propolis (8.29%), whereas the Toruń propolis and Łódź propolis belonged to different Tukey groups. The value for the Kraków propolis was close to the 5.8-6.6% range reported by Feás et al. (2014), whereas the remaining samples contained more water. Previous Polish data also indicate substantial variability, with values ranging from approximately 1% to more than 10%, depending on sample history and methodology (Kędzia 2009; Majewska and Trzanek 2009).

Moisture is affected by environmental exposure before collection, the proportion of hygroscopic plant material and pollen, particle size, harvesting conditions and storage. Because the samples were frozen after collection, the differences observed here most likely reflect the condition of the materials at harvest and their earlier handling rather than prolonged storage during the study. The combination of low wax and low moisture therefore makes the Kraków sample particularly favourable as a raw material.

Ethanol-soluble and insoluble fractions

The proportion of insoluble matter ranged from 31.61% in the Kraków propolis to 39.42% in the Łódź propolis, while the complementary ethanol-soluble fraction ranged from 60.58 to 68.39% (Table 2). The Kraków and Katowice propolis contained significantly less insoluble material than the samples from Warsaw, Legionowo, Łódź and Toruń and, consequently, had significantly higher calculated soluble fractions. The difference between the extreme samples was 7.81 percentage points, corresponding to approximately 13% more soluble material in the Kraków sample than in the Łódź sample when expressed relative to the latter value.

The results are consistent with physicochemical data reported for Portuguese propolis. Dias et al. (2012) found soluble fractions of 60.7-71.1% and insoluble fractions of 27.0-45.1%, while Feás et al. (2014) reported 60.2-67.3% soluble matter. The close agreement suggests that the extractability of the investigated Polish urban propolis is within the range expected for European propolis. Nevertheless, differences between studies may arise from solvent-to-solid ratio, ethanol concentration, extraction time, temperature, agitation or sonication and the procedure used to dry and weigh the residue.

The ethanol-soluble fraction is a useful technological indicator because it approximates the amount of material that can be recovered under defined extraction conditions. In European poplar-type propolis, this fraction is expected to contain phenolic acids, flavonoids and other resin-derived constituents, together with smaller amounts of co-extracted non-phenolic compounds. The insoluble residue may contain wax-associated particles, pollen, plant fragments, hive debris and resin components that are not efficiently solubilised by 70% ethanol (Bankova 2005; Pavlovic et al. 2020). Therefore, a high soluble fraction indicates favourable extractability.

Considering all of the measured parameters together, the Kraków sample set showed the most favourable physicochemical profile within the analysed materials: it had the lowest wax content, the lowest moisture content, the lowest insoluble fraction and the highest soluble fraction. The Katowice sample set also showed comparatively high extractability. In contrast, the Warsaw sample set combined the highest wax and moisture contents, whereas the Łódź sample set had the largest insoluble fraction. These observations characterise three independently collected composite batches from one apiary per city during the 2019 collection season and should not be extrapolated to all apiaries or all propolis from the respective cities without additional sampling.
Comparison with current specifications and study limitations

ISO 24381:2023 provides a current reference framework for raw Apis mellifera propolis and specifies, among other parameters, a minimum of 30% ethanol extractables on a dry-matter basis and a maximum loss on drying of 10%. However, the ISO procedures are not identical to those used in the present study: ISO determines ethanol extractables using 80% ethanol with prolonged extraction at 50°C and evaluates loss on drying at 105°C, whereas the present work used 70% ethanol with 20 min sonication and an oven-drying procedure at 80°C. The ISO standard also quantifies petroleum-ether extractables rather than the density-separated wax fraction used here. Consequently, the present data can be discussed in the context of ISO 24381:2023, but they should not be interpreted as a formal conformity assessment. A concise comparison with literature values and the ISO framework is provided below in Table 3.

Table 3. Comparison of the present physicochemical results with selected literature values and the ISO 24381:2023 framework.

Parameter	Present study	Selected literature	ISO 24381:2023 context
Wax / non-polar fraction	9.22-15.26% wax	Brazil: 2.3-16.4% wax (Negri et al. 1998); Portugal: mean 10.7% wax (Feás et al. 2014); South Italy: mean 27.98% wax (Grassi et al. 2023)	No direct equivalence. ISO uses petroleum-ether extractables (max. 65% for brown propolis), which include waxes and other non-polar compounds.
Moisture / loss on drying	5.66-10.34% moisture	Portugal: 5.8-6.6% (Feás et al. 2014)	Maximum 10% loss on drying. Direct conformity cannot be assessed because the ISO drying procedure differs from the present method.
Ethanol-soluble fraction	60.58-68.39%	Portugal: 60.2-67.3% (Feás et al. 2014); 60.7-71.1% (Dias et al. 2012)	Minimum 30% ethanol extractables on a dry-matter basis. Direct conformity cannot be assessed because ISO uses 80% ethanol and an exhaustive extraction procedure.
Ethanol-insoluble fraction	31.61-39.42%	Portugal: 27.0-45.1% (Dias et al. 2012); 36.4-40.0% (Feás et al. 2014)	No separate limit specified; it is complementary to extractable matter only under a defined analytical procedure.

A limitation of the present study is that each city was represented by one apiary and sampling covered a single collection season. Nevertheless, each apiary was represented by three independently collected composite batches obtained on separate sampling occasions at approximately two-week intervals. These

batches were treated as the replicate units in the statistical analysis. Pooling propolis from several available hives within each batch means that hive-to-hive variability cannot be estimated, and the use of one apiary per city does not allow city-wide variability to be characterised. In addition, one season cannot capture interannual changes in vegetation, colony management or harvesting conditions. The results should therefore be interpreted as comparisons among the sampled apiaries and their 2019 batches rather than as a survey of the current state of urban propolis in the respective cities. The present manuscript reports previously unpublished raw-material measurements from the same broader research campaign in which extract composition and antioxidant activity were subsequently published by Pobiega et al. (2023). Future work should include multiple apiaries per city, hive-level sampling where feasible, repeated collection years and harmonised methods, preferably including those specified in ISO 24381:2023.

Conclusions

Raw propolis from six Polish urban apiaries showed marked variation in colour, consistency, wax content, moisture content and ethanol-soluble and insoluble fractions. Within this 2019 sample set, the Kraków material combined the lowest wax and moisture contents with the highest soluble fraction, while the Katowice material also showed comparatively high extractability. These characteristics support their selection for further extraction and bioactivity studies, but they should not be interpreted as representative of all propolis from Kraków or Katowice. Validation using independently collected hive-level samples, additional apiaries and multiple collection years is required before broader conclusions about urban location or raw-propolis quality can be drawn.

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