

Quality Criteria for Propolis Standardization

Laura Stan, Liviu Al. Mărghițaș, Daniel Dezmirean

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca - 400372 Cluj-Napoca,
Calea Manastur 3-5, Romania

Abstract

Propolis is a natural resin produced by honeybees with high biological value for human kind. Although propolis is intensively used in medicine, cosmetics and lately in food industry also, there is no quality standard (Romanian or European) for this specific bee product. This article presents the quality criteria for Romanian raw propolis (56 samples) as a collection of data obtained along the years through extensive qualitative and quantitative analysis. Data obtained for extraction yield ($59\% \pm 8\%$), wax content ($35\% \pm 8\%$), total flavonoids ($9\% \pm 3\%$) and total phenolics ($28\% \pm 7\%$), antioxidant activity against DPPH are presented.

Keywords: antioxidant activity, DPPH, flavonoids, quality standard of propolis, phenolic compounds, propolis.

1. Introduction

Propolis has so far two main users: bees and humans. Bees produce propolis for their own benefit: sealing tight their hive and “chemical weapon” against pathogen microorganisms [1]. In order to produce it, bees have already “screened” the plant sources that possess pronounced antimicrobial and antioxidant properties from which they collected the resins and mixed them with their own secretions (saliva, wax) [2]. As for human part, propolis has been used in traditional medicine for more than 2000 years [3], while scientific research regarding its chemical composition and biological activity started only about 30 years ago [4]. Since bees use the natural available vegetation to create propolis, there is a high variability in the composition of propolis [5]. Until now over 180 different compounds were identified in propolis [5, 6]. They belong to different chemical compound classes: phenolic acids, flavonoids (flavonols, flavanones, flavanols, dihydro-flavonols), aminoacids, minerals. Many literature data show high correlations between the quantity and quality of the afore-mentioned

groups of compounds and propolis’ biological activity [7, 8]. Quantitative methods for these active compound groups proved to be more informative from the propolis’ quality point of view than the quantification of individual compounds. It was also noticed that regardless the geographical origin and its composition, it still exhibits beneficial effects on human health [1]. Research quality improved in time, along with technology advances. In the last decade propolis and other bee products gained the interest of consumers and companies due to its high biological value proven through multiple effects on treatment and prevention also. In order to protect the consumers and the honest producers the necessity of a quality standard for propolis is obvious. Ideally, a quality standard should propose methods that are fast, low cost, accurate, reliable and reproducible. In the present there are only two standards for propolis: a Russian standard unfortunately unavailable and with outdated methods, and the Argentinian standard for propolis [9]. International Honey Commission is involved in collecting data from European research teams regarding the analytical methods, general compositional criteria for propolis quality. Meanwhile, researchers from Japan, Korea, China

* Corresponding author: Laura Stan, Email:
laurastan1000@yahoo.com

and Taiwan develop their own quality standards for propolis [10-12].

In this article authors synthesizes the methods applied in our laboratory for propolis research and the results obtained so far on an extensive analysis over 56 propolis samples. Based on these results a description of compositional characteristics of propolis found in Romania is realized.

2. Materials and methods

Propolis samples (56) were collected by scrapping the bee frames or by means of a propolis collector. They were all kept in freezer (-20°C) until analysis. Details about sample preparations and methods used were described elsewhere [6, 13-16]. Chemical reagents: Ethanol absolute (Riedel-de-Haën, Germany), Methanol, AlCl₃, KOH, p.a., 2,4-dinitrophenylhydrazin p.a. were aquired from Merck, Germany, free radical DPPH (2,2-diphenyl-1-picrylhydrazyl, Aldrich Sigma, Germany), standards: Galangin (Fluka), Pinocembrin (Sigma-Aldrich), Caffeic acid, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carbonsäure, Fluka). Instruments: Spectrophotometric measurements were performed on Shimadzu Spectrophotometer UV-1700 equipped with 1cm quartz cells.

Propolis extraction. The method described already [6,13-16] by was used to obtain the Ethanolic propolis extract (1:100, w/v in Ethanol 70%). For every propolis sample 3 parallel extractions were performed by maceration (24h at room temperature with continuous agitation). This Ethanolic extract was evaporated to dryness and the resulted propolis balsam was further used to determine the antioxidant activity.

Quantitative determination of phenolics. The spectrophotometric method [6,14-16] for determination of flavones/flavonols, flavanones/dihydroflavonols, total phenolics was applied.

Antioxidant activity. Antioxidant activity of propolis was determined by 3 different methods based on DPPH: RSA, IC₅₀ and concentration in antioxidant compounds according to the method described by authors [6]. The violet solution of DPPH turns yellow in presence of antioxidants. The decrease of DPPH solution absorption value at 515 nm after addition of dry propolis extract was measured in a quartz cuvette (1cm).

3. Results and discussion

Romanian propolis presents the typical composition of poplar propolis found in temperate zone [2,6]. The chemical profile of poplar propolis can be characterized by three main parameters: total flavone and flavonol content, total flavanone and dihydroflavonol content, total phenolics content [1]. From the consumer point of view some extra issues must be taken into consideration. A propolis of good quality must have the following characteristics also: be free of toxic contaminants; contain acceptable low percentage of wax, insoluble matter and ash; be of a defined plant source determining the type of active compounds in it and to contain a high percentage of these active compounds [17].

Our team has already investigated in the last years the composition of Romanian propolis [6, 14, 15, 16]. This article presents an updated summary of the data obtained for Romanian propolis quality evaluation during the last 4 years in order to have a general characterization of this valuable bee-product.

The analyzed propolis samples presented 59±8% extraction yield of balsam and 35±8% beeswax. It was noticed a direct correlation between the harvesting method applied by the beekeeper to obtain propolis and the wax concentration in the sample. Therefore, the propolis samples collected by the means of hive tools had significantly higher amount of wax. Use of a propolis collector reduced the wax quantity below 21% which is an important issue regarding the good apicultural practices. There is a considerable amount of studies indicating that active dietary constituents of bee-products, fresh fruits, vegetables and beverages, prevent this free radical induced diseases and protect against foodstuff oxidative deterioration. These protective effects have been attributed to antioxidant species like vitamins, pigments, flavonoids and phenolic acids [7,8].

The flavonoids groups (Figure 1) determined in our samples had the following average values: 4±2% flavone/ flavonols, 5±2% flavanones/dihydroflavonols which are typical for poplar propolis [1, 2, 13]. Total phenolics (29±7%) were determined by Folin Ciocalteu method and they contain 9±4% total flavonoids and 20±7%.

phenolic acids. These results are similar to data presented by other authors about poplar propolis composition [1, 2, 13].

RSA values of $17 \pm 4\%$ were obtained for analyzed propolis samples and IC_{50} of $3 \pm 1\%$. There was noticed a positive correlation between concentration in total flavonoids and RSA values ($r^2=0,71$). Similar results [18] presented $r^2=0,5$ and RSA 10-80%, $r^2=0,762$ [7]. Concentration in

antioxidant compounds in propolis is directly correlated to quantity in phenolic compounds. The analyzed propolis samples presented 170mg caffeic acid/g propolis of concentration in antioxidant compounds.

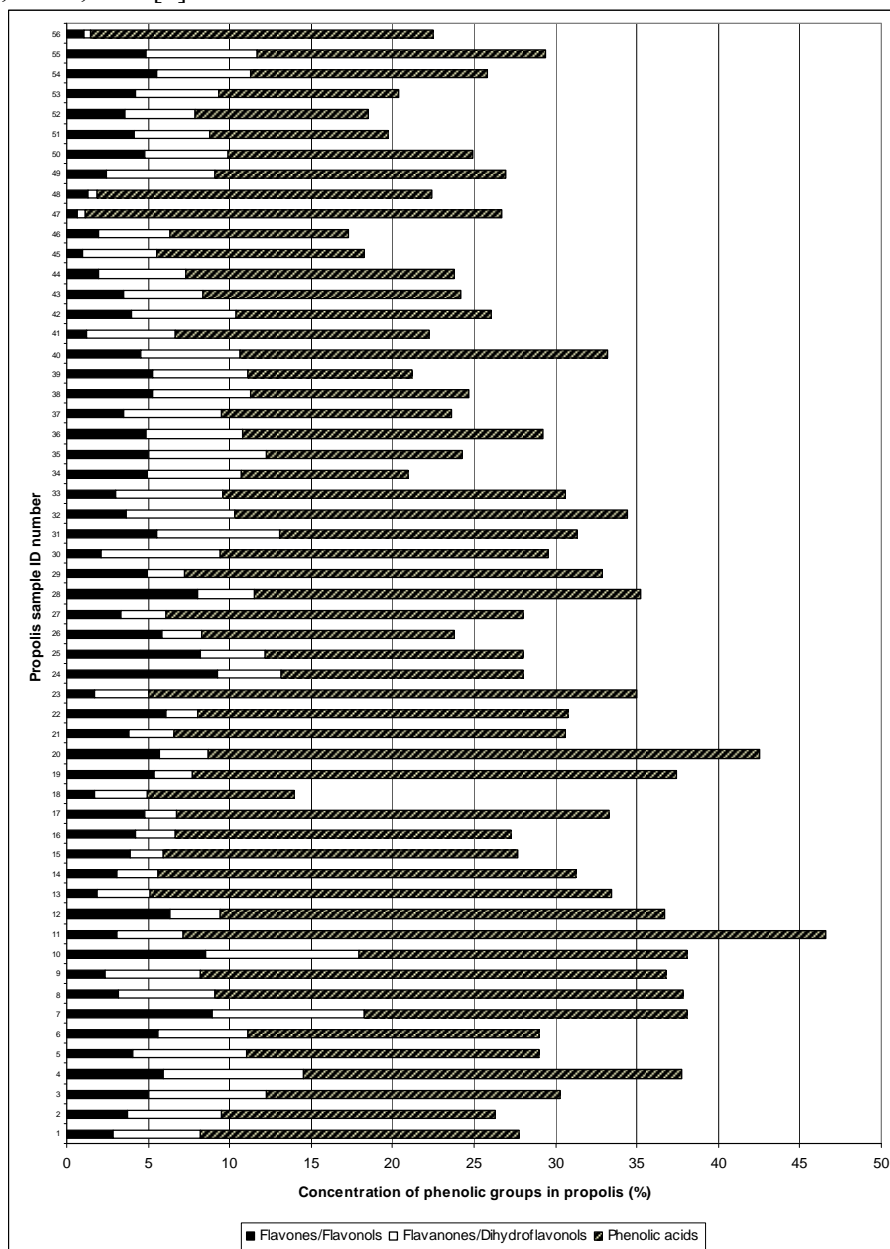


Figure 1. Concentration in total phenolics in Romanian propolis samples

4. Conclusions

Propolis has recently gained popularity all over the world as a natural valuable medicine and food additive for disease prophylaxis and health maintenance.

Increasing popularity of propolis all over the world draw attention to all segments of market:

producers (beekeepers - who increased their incomes), distributors (who created propolis based products), consumers (medical doctors, ill and healthy people, animals). Because it is so often used to promote health, it is important that the propolis made available is of high quality. Unfortunately, there still isn't available a certified system of quality control of propolis and products

based on propolis. Until a proper quality standard is developed, propolis will continue to be used as alternative treatment without the official acceptance in modern medicine.

The minimum values of the most important criteria for quality control of propolis are: total phenolics 20%, flavones/flavonols 4%, flavanone/dihydroflavonols 4%, balsam 35%, antioxidant activity (RSA 11%, IC₅₀ 1,5%, concentration in antioxidant compounds 170mg caffeic acid/g propolis). Authors wish to propose a maximum value for wax (40%) otherwise differentiation between adulterated and unadulterated propolis becomes difficult. Authors also wish to stress the importance of pollutants analysis and to recommend the lack of antibiotics, heavy metals, aflatoxines (B1, B2, G1, G2) in propolis samples used for medical purposes. Good apicultural practices should be applied by each and every beekeeper to obtain better quality of beeproducts in the hive. We have to remember that bees collect the beeproducts for their own benefit in the first place, and we as humans, are taking advantages of their hard work. If we take care to place the apiary in a location rich in food and material sources they need, then we have healthy bees contributing to pollination and biodiversity maintaining and other secondary aspects derived from these. The formulation of a quality standard for propolis is based on a large number of measurements. Therefore, further work is mandatory in order to cover all quality aspects of propolis and for statistical evaluation of data.

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