SUMMARY

BEE POLLEN CHARACTERISATION FOR FOOD SUPPLEMENTS PRODUCTION

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PhD thesis titled „ Bee Pollen Characterisation for Food Supplements Production", analyzes different pollen types from structural, physico-chemical and biological perspectives.
In addition, the nutritional potential and the capacity of pollen to develop api-nanosystems with improved biological characteristics are highlighted.

PhD thesis was structured in two principal parts, namely: part I, **Bibliographic study** and part II, **Own researches**, followed by exposure of bibliography and list of published scientific papers during thesis elaboration process.

The **Bibliographic study**is made up of two chapters, with a total of 37 pages, with a share of 21% of the thesis volume.

Chapter I (Morphological and physico-chemical characterization of bee pollen)refers to the morphological characteristics of pollen, highlights a number of macroscopic, microscopic and microstructural aspects and presents the chemical composition of pollen harvested by bees, treating individually the main classes of chemical compounds identified in pollen.

Chapter II (**Extraction methods used for separation and identification of bioactive compounds with nutritive and apitherapeutic value**) is devoted tothe extraction methods and conditions used for the separation of some nutritionally important compounds, but also stresses the nutritional and apitherapeutic value of pollen.

Part II includes own researches presented in 143 pages, representing 79% of the present paper, being structured in 5 chapters describing the purpose and objectives of the research, the vegetal material used, the natural geographic and institutional ranges in which they were carried out research, experimental results obtained and their interpretation, general conclusions and recommendations.

The research carried out aimed to establish the specific characteristics of each pollen types, to evaluate the biological activity, to obtain and characterize some nanostructured compounds with biomedical potential.

In order to the accomplish goal, the following objectives have been tabled and achieved the following aims:

- ✓ Morphological characterization of pollen types at macroscopic and microscopic level.
- ✓ Characterization of pollen types by identification and quantification of the main chemical compounds following the application of analytical methods.
- ✓ Elaboration and application of a chemometric method for differentiation of pollen type.
- ✓ The assessment of the main biological activities of pollen samples in vitro.
- ✓ The use of pollen extracts in achieving of nanostructured compounds.
- ✓ Characterization of the formed nanocomplex and evaluation of the biomedical potential of the nanostructured complex.

Each chapter of Part II contains a brief introduction, a description of the materials and methods used, results and discussions, and ends with a section intendedfor conclusionsfrom that chapter.

In **Chapter III**are presented the stages of sampling pollen specimens used for macroscopic, sensory characterization and the identification of botanical origin of pollen.

Chapter IV describes in detail the stages of physico-chemical characterization of bee pollen samples, representing the most voluminous experimental chapter.The following parameters were determined: humidity by thermogravimetric method, total protein content, free amino acid profile by liquid chromatography coupled with mass detector, mineral substances by calcination and ***optical emission spectrometry with inductively coupled plasma (ICP-OES)***, total sugars, free acidity, total lipids, fatty acids by gas chromatography, flavones and flavonols content, total polyphenols content, phenolic compounds profile by capillary electrophoresis technique, and application of FTIR ATR spectroscopy.The acquired IR spectra provided the database for bee pollendifferentiation and classification of samples by multidimensional statistical methods: Principal Component Analysis (APC), Linear Discrimination Analysis (ADL), and Partial Least Squares (PLS)Regression method.Statistical data was processed using Matlab (MathWorks, Massachusetts USA version R2015).APC and ADL were used to classify pollen according to botanical and / or geographical origin, while the PLS technique was applied to identify possible correlations between IR spectra and the main physico-chemical characteristics of pollen.

The FTIR-ATR spectra of 47 pollen samples were subjected to the chemometric analysis enabling of grouping pollen samples by botanical origin.Our experimental strategy combined the FTIR-ATR Spectral Analysis and the most advanced analytical methods, some of thesebeing a first of its kind at national level concerning chemical determinations on this work matrix. Also, in this chapter were described the pedo-climatic conditions in all the areas of apiary'slocations where the average rainfall and the monthly temperatures recorded during the study period were reported.

In **Chapter V**were presented the results of the evaluation of the main biological activities of bee pollen. Was envisaged also the determination of antioxidant activity using DPPH and ABTS radicals and antimicrobial activity on some clinical interest strains but also pathogenic microbial strains for some plant species.

Chapter VI presents the use of pollen extract to obtain inorganic-organic nanostructured compounds, their characterization and the demonstration of nanofluidstability. Investigation methods of nanostructured systems were dynamic light scattering, zeta potential measurements and electronic microscopy methods.Also in this chapter the synergistic combination of pollen extracts with Fe3O4-PABA magnetic nanoparticles was evaluated in order to identifying a hybrid nano-complex with superior antibacterial properties.

Chapter VII completes the thesis with the section of general conclusions drawn from the structural, chemical characterization and of use of pollen.

Results obtained

Chapter III - Bee pollen characterization after morphological and sensory aspects

Each macroscopic characterized of monochrome pollen sample was classified according to palynological analysis as a monoflorous sample (predominant botanical species> 90%), being identified 50% of the analyzed samples, bifloral (the presence of two botanical species) - 30% of the samples, or heterofloral (identifying three or more floral species in the pollen sample) - 20% of the samples included in the study.

The determination of botanical origin of all samples formed and performed by microscopic analysis identified 20 plant species belonging of 14 botanical families as follows:*Brassicaceae* (*Brassica sp*.), *Asteraceae* (*Carduus sp., Cirsium sp., Helianthus annuus, Solidago virgaurea, Matricaria sp.* şi *Taraxacum officinale*), *Fagaceae* (*Aesculus sp*.), *Plantaginaceae* (*Plantago lanceolata*), *Malvaceae* (*Tilia sp.*), *Rosaceae* (*Prunus L sp.* şi *Crataegus monogyna*), *Poaceae* (*Zea mays*), *Caprifoliaceae* (*Symphoricarpos rivularis*), *Vitaceae* (*Vitis sp*.), *Fabaceae* (*Trifolium repens*), *Papaveraceae* (*Papaver sp*.), *Umbeliferae* (*Apiaceae type*), *Lamiaceae* (*Lavandula sp*.), *Anacardiaceae (Anacardium sp.).*

The morphological analysis of the investigated pollen types identified the limits at the two poles of the microscopic dimensions at which the polar axis recorded values of 98.2 μm and 24.8 μm respectively, and the equatorial diameter of 97.9 μm and 25.4 μm respectively; these values occurring in pollen samples from *Zea mays* (maximum value) and *Brassica sp.* (minimum value).

Of all morphological parameters investigated under the optical microscope, the sculpture of the exine presents the most differentiations within the pollen types studied.Three of them present the echinate structure of the exine, all belonging to *Asteraceae* family. Striate and foveolate-striate sculptures respectively, were evidentiated in *Rosaceae* (*Crataegus monogyna* and *Prunus sp*.), and scabrate exine was found on *Plantago lanceolata* and *Papaver sp.* pollen species. The foveolate and

reticulate structure of the exine was identified of one type of the analyzed pollen types: *Tilia sp.* and *Brassica sp*., respectively.

Chapter IV - Physico-chemical characterization of monofloral bee pollen samples

The results obtained after water content determination of pollen samples demonstrate that this parameter is dependent on some weather indicators of the pollen harvesting period, such as temperature and precipitation.

The highest values in total protein content were recorded by floral species of the *Rosaceae* family (*Prunus sp.*and *Crataegus monogyna*), and from the general analysis of the data, pollens with a value greater than 20% are mainly derived from plant species with flowering during the spring period.In the composition of all bee pollen species, 8 essential amino acids were identified (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) and 14 non-essentials (histidine, arginine, asparticacid, alanine, glutamicacid, glutamine, glycine, proline, serine, tyrosine, ornithine, asparagine, 4-hydroxprolyne, sarcosine). The pollen of the Helianthus annuus species showed the highest value for the essential amino acids concentration, accounting for 27.5%, and over 90 percents indicated each of *Crataegus monogyna* and *Prunus sp*. for non-essential amino acids, relative to the total amino acids determined.

All pollen samples studied in this paper recorded the highest values for potassium concentration, the mineral content can be arranged in the following series: K>Ca>Mg>Fe>Zn>Mn>Cu>B.

As in the case of fatty acids we can talk about specificity among pollen species, the absence of fatty acids from different samples is observed in varying percentages: lauric acid is missing in about 60% of samples, margaric acid in more than 50% of samples, lignoceric acid is missing in over 44% of samples, while myristic and behenic acids were not identified in more than 20% and 10% of the analyzed samples, respectively.

In terms of flavone and flavonol content classification according botanical family, a higher content of these compounds was found in the species belonging to *Asteraceae* family, followed by the representatives of *Rosaceae* family; pollen from *Taraxacum officinalis* and *Helianthus annuus*showed increased levels of flavonoids and flavonols as well as total polyphenols content, indicating an important level of biologically active compounds in pollen samples.

Results of phenolic compounds screening indicated that 35% of the compounds analyzed were identified as unique components of the phenolic profile, and could contribute to the shaping of biological markers in the pollen types.

For botanical and geographical discrimination, was used the entire FTIR-ATR (4000-400 cm^{-1}) spectral range. The data obtained were used to identify a set of eight characteristic properties by applying the PCA-LDA-PLS techniques. After processing the first ten principal components (which represented 99,8% of the experimental variability), eight groups were separated in the

LDA1-LDA2.The obtained results recommend the FTIR-ATR spectrometry as a rapid method for identifying the botanical source in the case of unknown pollen samples and also for estimating the main physico-chemical properties.

Chapter V - The main biological activity of bee pollen

Prunus and *Brassica* species, along Papaver sp. and *Crataegus monogyna* showed the best antioxidant activity expressed by both the ABTS scavenger capacity and the DPPH method.

The antimicrobial activity of monofloral ethanolic extracts were assessed against Gram positive, Gram negative bacteria and fungi (strains of medical interest as well as pathogenic microorganisms for some plant species). Gram positive bacteria and fungi have been proved the most sensitive strains from those of clinical interest.

The study demonstrated the efficacy of all ethanol pollen extracts tested as strain inhibitors of *Xanthomonas campestris ICCF 274.*

Zea mays pollen extract showed less inhibitory activity against *Erwinia carotovora ICCF 138*strain compared to the other samples tested. Pollen extracts did not have antifungal effect against *Aspergillus niger* ICCF 92 strain.

Chapter VI - The use of bee pollen extracts for obtaining of some nonorganic-organic nanostructured compounds

Inorganic-organic hybrid nanostructured compounds were obtained by coating natural compounds of pollen extracts on Fe_3O_4 – PABA complex support. The characterization tests confirmed the presence of nano structures and indicated in some cases the parameters that setting out a minimum of predisposition to aggregation.

The confirmation of synthetized nanostructures sizes was done by electronic microscopy and their stability by Zeta potential. The hybrid nanostructures coated with api-compounds from pollen extract also confirmed their attachment by increasing the size of the obtained api-nanosystems.

Bee pollen api-nanosystem has shown inhibition of Gram positive and Gram negative micro-organisms. Among Gram positive bacteria, *S. aureus* strain showed to be the most sensitive to api-nanosystems functionalized with the following pollen extracts: *Brassica sp., Taraxacum officinallis, Plantago lanceolata* și *Zea mays,*while the same *Brassica sp*. pollen extract exerted the best inhibitory effect against Gram negative, *E. coli* strain.

The antifungal activity of the nanostructured hybrid complex has exhibited good inhibition of 50% of the tested strains, and of those, *C. albicans* demonstrated to be more sensitive to PABA/pollen api-nanosystem.

Originality elements

- Microscopic and physico-chemical characterization of pollen varieties derived from indigenous plants under climatic conditions of 2014-2015 years;
- Development and verified a chemometric method for identifying the botanical source of pollen and assessing the physico-chemical characteristics of bee pollen;
- Evaluation of biologic activity in vitro (antimicrobial activity) of pollen extracts against some vegetal pathogenic strains;
- Achieving inorganic-organic hybrid nanostructured compounds by coating natural compounds of pollen extract on complex support Fe_3O_4 – PABA;
- Testing the antimicrobial activity of pollen api-nanosystem.