

## OPINION

by **Prof. Ivan Iliev Atanasov, Agrobiointitute, AA**

of a dissertation for awarding the educational and scientific degree 'doctor' in the field of higher education 4. 'Natural sciences, mathematics and informatics', professional direction 4.2. 'Chemical Sciences', PhD program 'Organic Chemistry'. **Author: Assistant Professor Desislava Plamenova Gerginova. Topic: 'NMR metabolomics of honey and wine'. Scientific supervisors: Prof. DSc. Svetlana Simova and Ch. assistant professor Dr. Yavor Mitrev, Institute of Organic Chemistry with Center for Phytochemistry - Bulgarian Academy of Sciences (IOCCP-BAS)**

### 1. General presentation of the procedure and the doctoral student

By order No. RD-09-82 of 15.06.2022 of the Director of IOCCP-BAN, I have been appointed as a member of the scientific jury in the procedure for the defense of a dissertation on the topic "NMR metabolomics of honey and wine" for the acquisition of the educational and scientific degree "doctor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.2. 'Chemical Sciences', PhD program 'Organic Chemistry'. The author of the dissertation work is Assistant Professor Desislava Plamenova Gerginova - full-time doctoral student, and from 2021 an assistant at the Center for NMR Spectroscopy, IOHCF-BAS, with scientific supervisors Prof. DSc. Svetlana Simova and Ch. assistant professor Dr. Yavor Mitrev. The set of procedural materials presented by Assistant Professor Desislava Gerginova, including 4 publications on the dissertation work, is in accordance with the Regulations for the Development of the Academic Staff of the IOCCP, and meets the criteria of the IOCCP-BAS for the acquisition of the scientific and educational degree "Doctor".

Assistant Professor Desislava Gerginova was born in 1992. In the period 2011 - 2016, she completed the bachelor's program at the University of Chemical Technology and Metallurgy - Sofia, and in 2017 she completed the master's program and obtained a Master's degree from Sofia University "St. Kliment Ohridski". Since 2018, she has been enrolled as a full-time doctoral student at the Center for NMR Spectroscopy, IOCCP-BAS, and since 2021, she has been an assistant in the same research unit. In the framework of his scientific research activities so far, he has participated in a total of 19 projects and is the head of 1 project. Asistant Gerginova is the co-author of a total of 8 publications and 1 book chapter. In addition to the experimental experience related to MRI applications, Assistant Professor Gerginova successfully uses a wide range of general and specialized software necessary for processing the obtained experimental results. He speaks English and German.

### 2. Relevance of the topic

In recent years, a number of new studies in leading laboratories point to NMR as a major method with the potential for reliable and practical analysis of the composition, and determination of the quality and botanical and/or geographical origin of honey, as well as an effective method for the analysis of varietal and/or geographical origin of wines from traditional varieties. All this, together with the growing need to implement effective quality control of food products and wines and prevent fraud in this area, indicates that the subject of the dissertation is relevant both in terms of applying modern experimental approaches to the analysis of complex objects, as well as for the application of methods and approaches to solve problems important to society and the bioeconomy.

### **3. Knowing the problem**

Both the presented literature review and the entire content of the dissertation work and the attached publications indicate that Assistant Professor Desislava Gerginova has a thorough and excellent knowledge of the state of research and current issues on the topic of the dissertation. The literature review includes consideration of the development, main aspects and achievements of metabolomics as a part of 'omics' technologies, the principles and modern directions of development and applications of NMR analyses, as well as chemometric methods used in these studies. Along with this, the literature review also includes a detailed and analytical examination of the two main objects of research in the dissertation work: honey and wine, as well as products with a high sugar content. Overall, the information in the literature review is well structured, intelligently and creatively presented and interpreted, well illustrated with appropriate figures and tables of high quality, and fully corresponds to and supports the objectives of the dissertation.

### **4. Research methodology**

The set of analyzed samples, the methods used for sample preparation, NMR analysis and processing of the obtained results fully correspond to the topic and objectives of the dissertation work and fully contribute to the success of the conducted research. The experimental protocols and methods used are presented in detail in Chapter 4 'Experimental Part', grouped by purpose and objects of application.

### **5. Characterization and evaluation of the dissertation work and contributions**

The research in the dissertation includes the NMR analysis of three groups of objects: honey, wine and products with a high sugar content (mead and pine jam), the results of which are presented in three separate parts of Chapter 3 'Results and Discussion'. As a result of NMR analysis of 300 honey samples and appropriate processing of the obtained results, the doctoral student consistently established optimal parameters for honey analysis and a methodology for semi-quantitative determination of 41 components in honey based on the obtained <sup>13</sup>C NMR spectra. The following application of the established methods for NMR analysis of groups of honey samples of known origin and chemometric analysis of the obtained results allow correct differentiation of the analyzed types of honey in terms of /1/ botanical origin, incl. distinguishing honey from 16 different botanical species, and the complex analysis of the obtained results makes it possible to propose a 'decision tree' for determining the botanical origin of honey; /2/ entomological origin (honey from European bees /*Apis mellifera*/ and stingless bees /*Meliponini*/); /3/ geographical origin: nectar and honeydew honey from different countries and regions, including distinguishing the only Bulgarian honey with a protected

designation of origin - Strandzhan oak honeydew honey from other types of oak honeydew honey; and /4/ successful application of NMR analysis to establish dilution of honey and mixing with syrups. In addition to the specified groups of results, original results from this group of studies are /a/ the first establishment of the presence of the monosaccharide quinovose in bee honey; /b/ first <sup>13</sup>C NMR results for the presence of trehalulose in honey from African stingless bees; /c/ determination of the trisaccharides 1-kestose and 2,3-butanediol as characteristic of oak honeydew. In the framework of the second group of studies, an NMR analysis was carried out on 70 wine samples of known origin from different grape varieties, some of which were grown in different geographical areas or using different vinification processes. A chemometric analysis of the obtained NMR spectra of the analyzed samples allowed correct: /1/ differentiation of single-varietal wines obtained from different grape varieties, and as a result of the complex analysis of the obtained results, a "decision tree" was proposed allowing the differentiation of wines produced from 15 different grape variety; /2/ general grouping and differentiation of wines produced from Bulgarian grape varieties; /3/ differentiation of wines produced from the same varieties of grapes grown in different geographical regions of the country (wines with a protected geographical indication); /4/ distinguishing red wines from the same grape variety obtained as a result of different production processes without and with the use of maceration; /5/ as well as distinguishing diluted wines from real (undiluted) wines. The third group of studies includes original applications of NMR-based metabolomics to characterize the composition of samples of two types of pine jam and one type of mead and assess the compositional similarities between jam and honey, and mead and wine. Taken together, the mentioned research groups and the obtained results form three main achievements and the contribution of the dissertation work: /a/ a general methodological contribution including the accumulation of experimental techniques for NMR analysis and methods and approaches for processing large volumes of experimental NMR data, which allows the structuring of a set of methods and know-how for applying NMR-based metabolomics to the analysis of various types of complex samples and objects; /b/ a general scientific-applied contribution including the use of the accumulated data and know-how as a basis for further practical applications to assess the origin and quality of honey, wines and products with a high sugar content; /c/ general scientific contribution including obtained original scientific results for the presence of specific components in the composition of different types of honey. My overall assessment of the dissertation work is that it presents excellently planned and executed research involving NMR analysis of appropriately selected groups of samples and application of adequate and efficient processing of the obtained large volumes of experimental data for grouping and distinguishing the analyzed samples according to specific characteristics and NMR parameters. Here it is the place to point out that an undoubted merit of the dissertation is the presentation of the large volume of experimental results, and the main exposition includes excellently prepared and formatted colored figures with results of the grouping of the investigated samples and the proposed 'decision tree' for the individual types of samples. Besides the excellent visualization and interpretation of the obtained experimental data, such a presentation of the obtained results contributes to the popularization of the applications of NMR-based metabolomics and greatly expands the circle of readers who, despite not having experience in this field, would like to use this new 'omics' technology for specific research and problem solving from practice. The above gives me reason to once again express my excellent impression and to congratulate the doctoral student and her supervisors for the achieved results and the high quality of the dissertation work.

## 6. Evaluation of the publications and personal contribution of the doctoral student

Results of the dissertation have been published in a total of 4 scientific publications, three of which in scientific publications with IF/SJR (Molecules 2020 /Q1/, Foods 2021 /Q1/, Bulgarian Chemical Communications 2017 /Q4/, Journal of the Bulgarian Academy of Sciences 2021). The PhD student is first author in three of the publications and second author in one publication, which confirms her personal contribution to achieving the published results. In the available scientific literature, a total of 15 citations of the publications on the dissertation work have been found so far, which demonstrates the interest of other researchers in the published research and results. In addition to the mentioned scientific publications, results of the dissertation work were presented in a total of 5 reports at national scientific conferences, a total of 4 reports at international scientific conferences and a total of 9 posters presented at various scientific forums, 3 of which were awarded.

## 7. Abstract

The presented abstract is prepared according to the requirements and it fully presents the results, discussions and conclusions included in the dissertation work.

## 8. Recommendations for future use of dissertation contributions and results

In addition to the attractive possibilities for applications of the accumulated data and know-how for NMR analysis and assessment of the origin and quality of honey, wines and other products with a high sugar content, I believe that the results and accumulated know-how of the dissertation work can and should to be used for more detailed studies on the relationship between bee grazing and honey yield and composition. In this regard, I would like the PhD student to provide an opinion on how effectively NMR-based metabolomics can be used for the comparative analysis of flower nectar composition in individual plant species and the composition of the resulting monofloral honey, and to assess nectar utilization efficiency in the flowers of honey plants to obtain colored honey.

## CONCLUSION

The presented dissertation of the doctoral student Desislava Gerginova contains scientific, scientific and applied results, which represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of the ZRASRB and the Regulations for application of the National Academy of Sciences of the Bulgarian Academy of Sciences. The presented materials and dissertation results fully comply with the specific requirements of the Regulations of IOCCP-BAS for the application of ZRASRB.

The dissertation shows that the doctoral student Desislava Gerginova has in-depth theoretical knowledge and professional skills in NMR spectroscopy and applications of NMR-based metabolomics and, in general, in the scientific specialty of organic chemistry, demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my **positive assessment** of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and I propose to the honorable scientific jury **to award the educational and scientific degree "doctor" to Assistant Professor Desislava Plamenova Gerginova in field of higher**

**education 4. 'Natural sciences, mathematics and informatics', professional direction 4.2.  
'Chemical Sciences', PhD program 'Organic Chemistry'**

24.08. 2022



.....  
(Prof. DSc Ivan Atanassov)