

REVIEW

by **Prof. Dr. Stefan Leonidov Tsakovski**,

Faculty of Chemistry and Pharmacy, Sofia University “St. Kliment Ohridski”
member of a scientific jury approved by order RD-09-82 / 15.06.2022 of the Director of
the Institute of Organic Chemistry with Centre of Phytochemistry - BAS

On a doctoral thesis entitled “NMR metabolomics on honey and wine”
for awarding the educational and scientific degree 'doctor' e.g. PhD in
the professional field: 4.2. Chemical Sciences, Doctoral Program in Organic Chemistry

Author: **Dessislava Plamenova Gerginova**

Supervisors: **Prof. DSc Svetlana Simova**

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Dessislava Plamenova Gerginova obtained her BSc (Inorganic Chemical Technologies) in 2016 from the University of Chemical Technology and Metallurgy and MSci (Modern Spectral and Chromatographic Analytical Methods) in 2017 from the Faculty of Chemistry and Pharmacy, Sofia University “St. Kliment Ohridski”. Since 2014 she has started her professional career in the Institute of Organic Chemistry with Centre of Phytochemistry – BAS (IOCCP) as a technician; since 2018 she has been a full-time PhD student at the IOCCP and since 2021 she has been appointed as an Assistant Professor at the same Institute.

The doctoral thesis has been evaluated and directed for a defense from the Scientific Council of the IOCCP. The review of **the submitted materials** shows that the legal requirements for conducting the doctoral studies are met.

The main subject of the doctoral thesis under review are NMR metabolomics studies on honey and wine. The research focuses on quality control and verifying the authenticity of the listed foods. The presented study is in an intensively developing scientific area worldwide. Unfortunately, there are no systemic quality control studies in Bulgaria and verification of the Bulgarian foods and drinks' authenticity, including application of appropriate statistical methods. Therefore I estimate the research presented in the doctoral thesis as up-to-date and important for quality control and verification the authenticity of Bulgarian honeys and wines.

The doctoral thesis follows classical and logical structure and is well illustrated with **tables and figures**.

The **brief and concise introduction** logically leads to the goal, namely the development of NMR methods for determining the quality and authenticity of various types of honey, wine

and other products with a high content of sugars, as well as their inclusion in databases. To this aim **five scientific tasks have been correctly formulated**.

The literary review introduces the necessary information regarding the objects of research, but is too extensive (almost 50% of the doctoral thesis). It shows a good understanding of metabolomics as a research area and describes in detail the NMR spectroscopy, the applied chemometric methods and the investigated objects. I have **the following notes** to the presentation of the chemometric methods:

1. As a common knowledge multivariate statistical methods are considered those working with four or more variables. In this regard, the Correlation Analysis should not be considered in the group of the multivariate statistical methods.
2. In the literature review, the multivariate methods are correctly divided into two groups: (i) without predefined classes (Pattern Cognition) – Cluster Analysis and Principal Component Analysis, and (ii) with predefined classes (Pattern Recognition) – Partial Least Squares-Discriminant Analysis (PLS-DA) and its orthogonal version OPLS. In view of the different goals of the two groups approaches for patterns cognition and patterns recognition, the analysis of the ROC curve and the area under the curve (AUC) as well as the confusion matrix are the parameters used to evaluate the statistical models working dealing with predefined classes data. In this regard, to determine the number of components in the Principal Component Analysis, other parameters are usually applied (eigenvalue, % explained variation) rather than AUC.

The results of the doctoral thesis follow the formulated research tasks and are distinguished according to the studied objects:

1. Honey:
 - the optimal conditions and parameters for the analysis of bee honey by means of NMR have been established;
 - two models have been developed: PLS-DA (AUC>0.90) and decision tree (293 correctly classified out of a total of 296 samples), which can be used to predict the botanical origin of honey based on the 41 analyzed components and the sum of unidentified substances (U1-U16);
 - preliminary results for distinguishing honey according to its entomological origin are presented, which can be used as a basis for conducting future comparative studies;
 - models with an accuracy of 99 % (linear discriminant analysis (LDA), OPLS) have been developed to determine the geographical origin of the analyzed honeydew and floral honey samples;
 - a methodology and appropriate markers for identifying samples of diluted honey and syrups, as well as a method for calculating the optical rotation angle of honey, are proposed.
2. Wine:

- the optimal conditions and parameters for the analysis of wine by means of NMR have been established;
 - several classification models are presented with an accuracy higher than 98 % for distinguishing wines according to the grape variety used and the proposed decision tree enables the rapid identification of new samples;
 - cluster analysis and PLS-DA are proposed to distinguish genuine from diluted wines, describing the component profiles of the two groups;
 - a comparison was made of the chemical composition of wines produced with and without maceration;
 - an OPLS model has been developed for distinguishing typical Bulgarian wines from foreign varieties;
 - two PLS-DA models are presented distinguishing white and red wines made from grapes from the Danube Plain and the Thracian Lowland.
3. Other products with a high sugar content:
- ¹H NMR analysis of mead has been performed and a comparison of the detected substances with those in wine and honey samples has been presented;
 - the sugar profile of pine jam has been determined and compared with the sugar profiles of honey samples.

The discussion of the above-presented scientific results is comprehensive and correctly written. The conducted analysis, the comparative representation of the results in relation to the available knowledge in the field of the doctoral thesis, the logical and correctly formulated hypotheses demonstrate a good knowledge of the problem and well-developed scientific capacity.

The conclusions correctly summarize the presented results and analyses.

I estimate **the contributions** of the doctoral study as such with fundamental and applied scientific character.

I have no critical comments on the content of the work, but I would like to give the following **recommendations** to Assist. Prof. Dessislava Gerginova for further metabolomic studies:

1. It is recommended in the presentation of multivariate statistical models to indicate, in addition to the validation approaches used and the main statistical parameters, the size of the input data matrix (numbers of samples and variables) and the input data transformation used.
2. It is appropriate in the figures presenting the concentration profiles of given classes (Nightingale diagram, box plot) to indicate the statistically significant differences assessed by the appropriate statistical tests.
3. It is desirable to use external validation (prediction) during developing of classification models even for part of the studied sample classes.

4. The selection of the variables (components) prior PLS-DA by means of ANOVA is not necessary. It is better to use the VIP parameter and/or the selectivity ratio if the selection of variables is recommended.
5. The contributions of the individual components in the presented PLS-DA models are better to be represented by a heat map obtained by the simultaneous clustering of samples and component contributions.

The publication activity of Dessislava Gerginova includes four research papers, three of which are in journals with an impact factor. It should be noted that the publications cover the main doctoral results. In addition, in three of them, the PhD student is the first author reflecting her key role in the conducted research. The publications have already 15 citations that is a good attestation of their quality. The PhD student's experience includes also twenty participations in scientific forums. Further the PhD student has demonstrated active participation in research projects, which is a prerequisite for successful career development. Assist. Prof. Dessislava Gerginova has participated in a total of 18 national and international scientific projects, and she has been also a leader of a project "¹H NMR profiling of mead" in the frame of the National Program "Young Scientists and Postdoctorants"

In **conclusion**, I find that the doctoral thesis of Assist. Prof. Dessislava Gerginova presents a competent and thoroughly conducted study with original scientific contributions. The obtained results and the publication activities meet and exceed the requirements of the Development of Academic Staff in the Republic of Bulgaria Act, the Rules for its implementation and the Internal Rules of the Bulgarian Academy of Sciences and those of the Institute of Organic Chemistry with Centre of Phytochemistry – BAS.

The **attached author's abstract** adequately reflects the content of the doctoral thesis.

The above gives me a reason to provide **a positive assessment** and to propose to the scientific jury to award Dessislava Plamenova Gerginova the educational and scientific degree "Doctor" in the professional field: 4.2. Chemical Sciences (Doctoral Program in Organic Chemistry).

24.08.2022

(Prof. Stefan Tsakovski)