OPINION

by Assoc. Prof. Dr. Tonka Atanasova Vasileva,
Department of Biochemistry and Microbiology
University of Plovdiv "Paisii Hilendarski"

of a dissertation for the award of the educational and scientific degree **"doctor"**
in: field of higher education 4. Natural sciences, mathematics and informatics,
professional field 4.3 Biological sciences,
doctoral program Biochemistry

Author: Teodora Mincheva Panayotova

Topic: *"Investigation of the properties of biologically active peptides obtained by enzymatic hydrolysis using proteolytic enzymes from lactic acid bacteria"*

Scientific supervisor: *Prof. Dr. Iliya Nikolov Iliev - Plovdiv University "Paisiy Hilendarski"*

1. General presentation of the procedure and the doctoral student

By order No. PD-22-742 of 07.04.2026 of the rector of Plovdiv University "Paisii Hilendarski", I have been appointed as a member of the scientific jury for ensuring a procedure for the defense of a dissertation on the topic "Investigation of the properties of biologically active peptides obtained by enzymatic hydrolysis with proteolytic enzymes from lactic acid bacteria" for the acquisition of the educational and scientific degree "doctor" in: field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3 Biological sciences, doctoral program Biochemistry. The author of the dissertation is Teodora Mincheva Panayotova - a part-time doctoral student at the Department of Biochemistry and Microbiology, with scientific supervisor Prof. Dr. Iliya Iliev from PU "Paisii Hilendarski".

The set of electronic materials presented by Teodora Panayotova is in accordance with Art. 36 (1) of the Regulations for the Development of Academic Staff at Plovdiv University, and includes the following documents: an application to the Rector of Plovdiv University to initiate the procedure for the defense of the dissertation; a CV in European format; minutes of the department council, related to reporting the readiness to open a procedure and preliminary discussion of the dissertation; the dissertation; an abstract in Bulgarian and English; a list of scientific publications on the topic of the dissertation; copies of the scientific publications; a declaration of originality and authenticity of the attached documents; a statement of compliance with the national minimum requirements for acquiring the educational and scientific degree "doctor".

The doctoral student has presented three scientific publications on the topic of the dissertation, published in refereed scientific journals.

Teodora Panayotova graduated with a Bachelor's degree in “Meat and milk technology“ in 2010 and a Master's degree in ”Control and safety of food of animal origin" in 2011 at the University of Food Technologies, Plovdiv. From 2012 to the present, Teodora Panayotova has been working as a researcher at “LB Bulgaricum” EAD, Sofia.

Teodora Panayotova was enrolled as a part-time doctoral student on March 1, 2020 in the Department of Biochemistry and Microbiology at the Faculty of Biology, Plovdiv University, and in 2025 she was granted the right to defend her thesis..

2. Relevance of the topic

Milk proteins are a rich source of low-molecular-weight peptides with certain biological activities (antioxidant, antihypertensive, antimicrobial, immunomodulatory, etc.), which are released during digestion, during food processing, during controlled enzymatic hydrolysis, as well as during fermentation of milk proteins by lactic acid bacteria. The growing need for food for the world population and the increasingly common allergies to proteins of animal origin are a prerequisite for the use of plant proteins to obtain biologically active peptides. The antihypertensive properties of peptides obtained by hydrolysis of proteins from some plants are of significant scientific interest, in terms of their ability to inhibit the activity of angiotensin converting enzyme (ACE). As a part of the renin-angiotensin system, ACE is a major target for pharmacological inhibition and regulation of blood pressure. In this regard, I consider that the topic of the presented dissertation work on the production of bioactive peptides with inhibitory activity against ACE when cultivating lactic acid bacteria in nutrient media with plant proteins is relevant and has scientific and applied significance.

3. Knowing the problem

The information presented in the literature review is systematized on 37 standard pages, including 15 figures, 5 tables and 2 appendices. The doctoral student has presented the characteristics of lactic acid bacteria, emphasizing their proteolytic system, which is directly important for obtaining biologically active peptides. Sources and basic methods for obtaining low-molecular peptides, as well as their physiological properties, are examined in detail. Literature data from 324 literary sources are summarized, which testifies to the theoretical preparation of Teodora Panayotova on the topic of the dissertation. Based on the literature analysis, the doctoral student clearly justifies the purpose of the study and formulates 5 specific tasks for its achievement.

4. Research methodology

The research methodology has been selected in accordance with the set goal. The presented methods are a prerequisite for the successful implementation of the experimental tasks and the reliability of the obtained results. In the course of the experimental part of her work, the doctoral student has mastered classical and modern methods, which include: microbiological and biochemical methods, including quantitative, enzymatic, electrophoretic analyses and chromatographic techniques. The methods are described in detail and I believe that Teodora Panayotova has acquired the necessary methodological experience in conducting the experiments.

5. Characteristics and evaluation of the dissertation work and contributions

The presented dissertation is structured according to the requirements for such a type of work and is written on 182 standard pages with the following sections: Introduction; Literature review; Aim and objectives; Materials and methods; Results and discussion; Conclusions; Contributions; Applications; References. The dissertation work presents a study of 50 strains of lactic acid bacteria of the species *Lactobacillus delbrüeckii* subsp. *bulgaricus*, *Lactobacillus helveticus*, *Lacticaseibasillus casei* and *Lactiplantibacillus plantarum* to secrete proteolytic and aminopeptidase enzymes when cultivated in the presence of various plant proteins, as well as optimizing the conditions for obtaining biologically active peptides inhibiting the activity of ACE from selected strains of lactobacilli. The experimental work is systematized in 5 separate parts, which logically follow the tasks set. The results obtained from the conducted studies are illustrated in 28 figures and 4 tables. Each individual experimental part ends with an analysis and summary of the most important results achieved. The above proves the methodological and theoretical skills of the doctoral student in conducting the experiments and interpreting the obtained results, which I consider convincing and reliable. The seven conclusions formulated correspond to the tasks set and faithfully reflect the obtained results. Four original scientific-applied and applied contributions have been formulated, which reflect the main achievements of the dissertation work.

6. Assessment of the publications and personal contribution of the doctoral student

Three scientific publications have been presented in connection with the dissertation - two published in *Acta Microbiologica Bulgarica* (Q4) and one published in *Bulgarian Chemical Communications* (Q4). In all publications, the doctoral student is the first author, which testifies to her leading participation in processing the results and preparing the materials for publication. The doctoral student has participated in two national scientific forums with international participation and one international symposium. One of the publications presented on the dissertation has been cited twice in refereed scientific publications (category Q1), which is an indicator of the scientific interest and recognition of the published results.

I consider that the research conducted and the contributions formulated in the dissertation are the result of the work of Teodora Panayotova, under the supervision of her scientific supervisor, Prof. Iliya Iliev.

7. Abstract

The abstract is prepared according to the requirements and faithfully reflects the goal, objectives, main results achieved, formulated conclusions and contributions of the dissertation work.

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

I recommend that the work of Tedora Panayotova continue with optimizing the process for quantitatively obtaining biologically active peptides with ACE-inhibitory activity from selected lactobacilli strains, studying their amino acid composition and obtaining a standardized industrial product.

9. Critical remarks, recommendations and questions.

I have the following question for the doctoral student:

What stages of additional research would allow for controlling the process associated with obtaining biologically active peptides from the studied lactobacilli strains and its implementation in production?

CONCLUSION

The dissertation contains scientific and applied results that represent an original contribution to science and meet the requirements of the Low on the Development of Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the relevant Regulations of Plovdiv University “Paisii Hilendarski”.

The dissertation shows that the doctoral student Teodora Mincheva Panayotova possesses the necessary theoretical knowledge and professional skills in the scientific specialty of Biochemistry, demonstrating skills for independently conducting scientific research.

In view of the above, I give my positive assessment of the conducted research, presented by the above-reviewed dissertation, abstract, achieved results and contributions, and I propose to the scientific jury to award the educational and scientific degree "Doctor" to Teodora Mincheva Panayotova in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences, doctoral program in Biochemistry.

18.05.2026 Opinion by: …….....

(Assoc. Prof. Dr. Tonka Vasileva)