

## REVIEW

By: Prof. Iskra Vitanova Ivanova, PhD

Subject: evaluation of dissertation work for the acquisition of the educational and scientific degree "Doctor" in the field of higher education "Doctor", Field of Higher Education: 4. Natural Sciences, Mathematics and Informatics Professional field: 4.3. Biological Sciences; Doctoral program: Biochemistry

Author of the dissertation work: Teodora Mincheva Panayotova

Title of the dissertation work: "Investigation of the properties of biologically active peptides obtained by enzymatic hydrolysis with proteolytic enzymes from lactic acid bacteria"

Scientific supervisor: Prof. Dr. Iliya Nikolov Iliev

By order No. PD-22-742 of 07.04.2026 of the Rector of Plovdiv University "Paisiy Hilendarski" (PU), I was appointed as a member of the scientific jury.

### 1. RELEVANCE AND SIGNIFICANCE OF THE DEVELOPED PROBLEM

Peptides obtained from natural sources represent a rich and versatile class of bioactive compounds with enormous potential in biomedicine, biotechnology and food sciences. Their versatile ability to exert antimicrobial, antiplatelet, antioxidant and antitumor effects positions them as indispensable tools in the pursuit of innovative therapies and high-quality functional products. However, their full-scale use continues to face significant hurdles in terms of industrial production, metabolic stability, and systemic bioavailability. The synergy of technological advances in proteomics, bioinformatics, chemical synthesis, and recombinant production has effectively transformed these limitations into strategic opportunities. These integrated platforms allow for precise optimization of the activity and safety of peptides, facilitating their successful transition into various industrial sectors. Furthermore, the application of multidisciplinary approaches, including artificial intelligence and nanotechnology, promises to significantly expand their therapeutic scope. Future research in the field should prioritize the development of sustainable, scalable manufacturing models and the design of multi-target peptides with improved controlled-release systems. Rigorous *in vivo* efficacy and safety evaluation, along with deep integration of multi-omics and artificial intelligence-driven tools to accelerate product discovery and screening, will be essential for this progress. By addressing these priorities, natural peptides will solidify their role as fundamental pillars in personalized medicine and functional nutrition, providing more effective and environmentally friendly alternatives. With promising prospects, these biomolecules continue to pave the way for a new era of innovation and sustainability in life sciences. Plant-derived materials represent one of the most extensively studied sources of biologically active peptides. Common plant sources include soybeans, rice bran, sesame, nuts, wheat, corn gluten. Peptides generated from these materials are often associated with probiotic-supportive effects, in particular promoting the growth of *Lactobacillus* and *Bifidobacterium*.

All this gives me reason to assess the presented scientific work as up-to-date, with the potential for scientific achievements that can have a rapid practical implementation.

The materials presented by the doctoral student fully meet the requirements for the defense of the doctoral thesis and present the specific evidentiary part regarding the required criteria, as well as present the overall production of the candidate. The dissertation is presented on 182 standard pages of text. The generally accepted scheme and the recommended ratios between the individual parts of the work have been observed, as follows: Introduction – 1 page; Literature review – 36 pages; Aim and objectives – 1 page; Materials and methods – 19 pages; Results and discussion – 71 pages; Conclusions 2 pages. The results obtained are illustrated with 52 figures, 7 tables, 2 appendices and 324 literary sources.

## 2. LITERARY AWARENESS AND STATEMENT OF THE GOAL AND TASKS

This dissertation is complex and assumes a good knowledge of the literary sources and the methods for solving it. The doctoral student has made a comprehensive review of the achievements of other researchers, which she was able to convey and analyze on 36 pages in the literature review. The review presents the state of the problem in detail and proves the need for the development of a brief characterization of lactic acid bacteria and their proteolytic system. The author sequentially reviews the data on the proteolytic system, which consists of extracellularly located proteinases, transport systems specific for di-tripeptides and oligopeptides (> 3 residues), and numerous intracellular peptidases. Peptides generated by the action of cell-associated proteinases released into the extracellular environment and their penetration into the cell by various transport systems, such as oligopeptide permease (Opp), transporting peptides with 4 to 18 amino acid residues, and transport systems for the transfer of di- and tripeptides, are considered. Protein degradation by cell-associated proteinases, newly produced oligopeptides are taken up by the oligopeptide transport of the cells and hydrolyzed to lower molecular weight peptides and amino acids by the concerted action of peptidases with different and partially overlapping specificities. Currently, more than 1500 different biological peptides have been reported in a database under the name "Biopep". They show hormone- or pharmaceutical-like activity and can be classified based on their mode of action as antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral-binding and antioxidant. The author examines the relationship between sources of bioactive peptides isolated from plants and the increased demand for food to meet the needs of a rapidly growing world population. Cow's milk, cheese and dairy products are the largest sources of bioactive proteins and peptides derived from food.

Methods for isolating bioactive peptides by enzymatic hydrolysis using proteolytic enzymes of the digestive system, microbial fermentation, chemical and/or physical methods are presented. To increase the degree of hydrolysis in the generation of bioactive peptides, new approaches are presented, such as microwave, ultrasonic extraction, ohmic heating, pulsed electric fields, subcritical water extraction.

The literature review is specific, structured correctly, and follows the logical connection of the information. The data from the reference served for the clear and correct definition not only of the goal, but also for the formulation of the tasks. The literature (both in the review and in the entire work) is closely related to the topic of the dissertation. The literature list includes an impressive number of 324 titles in Latin. They are mainly from recent years. This speaks of the excellent theoretical awareness of the doctoral student and the aim of finding a new scientific challenge.

### 3. EVALUATION OF THE METHODS AND MATERIALS USED

The aim of this dissertation is to investigate the production of biologically active peptides with ACE-inhibiting activity when cultivating lactic acid bacteria in nutrient media with vegetable proteins. 5 well-founded experimental tasks are set for solving. The "Materials and Methods" section demonstrates an impressive set of methods, tailored to the specific requirements of the experiments. They are modern and adequate for the implementation of the dissertation work. They are described accurately and in detail, fully covering the multifaceted areas of work: from classical to modern research. Microbiological and biochemical methods were used, including determination of enzyme activity, polyacrylamide electrophoresis and ultra-high-performance liquid chromatography with mass spectrometry (UHPLC-MS), etc.

All this allows me to give high marks to the scientific level and the excellent preparation, which manages to correctly combine a variety of classical and modern methods for the purposes of the dissertation, successfully solving the experimental tasks set.

### 4. EVALUATION OF THE OBTAINED RESULTS

The section "Results and Discussion" is well structured, supported by tabular and graphical material, with an appropriate interpretation of the results obtained by foreign scientific teams. The author consistently presents evidence for her scientific thesis, thus logically finalizing experimental work.

A large-scale and diverse experimental work was carried out within the framework of a complex biochemical study. Based on an initial screening of 50 strains of lactobacilli, belonging to four species *Lactobacillus delbr. ssp. bulgaricus* (30), *Lactobacillus helveticus* (11), *Lactocaseibacillus casei* (4) and *Lactiplantibacillus plantarum* (5), the following were selected: with the highest proteolytic activity the strains *Lactobacillus helveticus* AB, and one *Lactocaseibacillus casei*. . Pea protein hydrolysate, soy protein hydrolysate and whey protein WP 80 to the highest extent activate the proteolytic system in the selected lactobacilli strains. Specific aminopeptidase activity was established when studying four aminopeptidases – leucine aminopeptidase, lysine aminopeptidase, arginine aminopeptidase and proline aminopeptidase from the selected lactobacilli strains. The studied strain *Lactobacillus helveticus* b244 has the highest ACE inhibitory activity when cultivating a nutrient plant medium with soy protein and pea protein. The highest ACE inhibitory activity was obtained in an all-vegetable nutrient medium containing 5% soy protein and 2% glucose. Strain *Lactobacillus helveticus* b244 showed the highest values of ACE inhibitory activity in all tested media. After SDS-PAGE tris-tricine electrophoresis, it was found that the selected strains *Lactobacillus helveticus* h70 and *Lactobacillus helveticus* actively hydrolyze not only milk proteins, but also protein fractions in nutrient media containing vegetable proteins. Electrophoretic analysis confirms that the strains *Lactobacillus helveticus* h70 and *Lactobacillus helveticus* b244 cultivated in a nutrient medium with pea protein present low molecular weight peptides < 6.5 kDa. The conducted ultra-high performance liquid chromatography with mass spectrometry (UHPLC-MS) detects low molecular weight peptides when cultivating strains *Lactobacillus helveticus* h70 and *Lactobacillus helveticus* b244 in a vegetable nutrient medium with pea protein. . Combined nutrient media containing milk and vegetable proteins (pea and soy protein) have a synergistic effect and contribute to the emergence of new peptide fractions after fermentation with strains *Lactobacillus helveticus* h70 and *Lactobacillus helveticus* b244.

The obtained results presented in "Results and Discussion" logically follow the course of solving the tasks set. They are summarized and discussed in the light of published data from recent years.

Both the idea and the volume of research conducted on the implementation of this task and throughout the work deserve high praise. The discussion of each experiment, the comparison of the results and the comparison with the literature data, once again emphasize the qualities of the doctoral student in mastering experimental theory. With this, she proves that she has fully mastered the third stage of her training and is a complete experimenter.

#### 5. CONTRIBUTIONS AND SIGNIFICANCE OF THE DEVELOPMENT FOR SCIENCE AND PRACTICE, REMARKS AND QUESTIONS

I accept the contributions made.

I consider the presented data proving the production of bioactive peptides with ACE inhibitory activity when cultivating selected strains of lactobacilli on nutrient media containing pea and soy protein to be particularly significant. For the first time, two new plant nutrient media containing pea and soy protein have been formulated, suitable for cultivating selected strains of lactobacilli, which will be easily applicable in production processes.

Teodora Panayotova is the author of 3 scientific publications, in three of which she is a leading researcher and three participations in scientific forums, which shows her creative and research activity in their development and shaping.

I have a few questions for the dissertation:

- ☐ Can biologically active peptides with a specific amino acid sequence be produced selectively through existing enzymatic or microbial fermentation methods, and not through chemical synthesis?
- ☐ What are the most used methods for analyzing the structure of peptides?

#### CONCLUSION

The topic is relevant, the doctoral student has mastered modern methods, the experiments are methodically correct, the results obtained are reliable and are a solid basis for further scientific and applied developments. Exceptionally original scientific and applied contributions stand out.

Based on the above, I can confidently state that the peer-reviewed dissertation is an original scientific development, with theoretical and applied significance. The proposed dissertation is evidence that Teodora Panayotova has developed competencies necessary for the award of the doctoral degree, including theoretical preparation, methodological knowledge, independence and experience in planning experiments and the ability to analyze results. The dissertation contains scientific, scientifically applied and applied results that represent an original contribution to science and meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADSRB), the Regulations for the Implementation of the ADSRB and the relevant Regulations of the University "Paisiy Hilendarski" and I give my high assessment and recommend to the members of the scientific jury to award the

dissertation candidate TEODORA MINCHEVA PANAYOTOVA the educational and scientific degree "Doctor" in professional field 4.3. Biological Sciences, Specialty Biochemistry.

15.05.2026 г.

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