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Review
of the doctoral thesis of Mr. Antoni Taraszkiewicz, M.Sc., Eng. entitled:
"Characterization of bioactive peptides from chicken feather keratin and products of their transformations occurring during the Maillard reaction"

The doctoral thesis submitted for review was prepared at the Department of Chemistry, Technology and Biotechnology of Food, Faculty of Chemistry, Gdańsk University of Technology, under the supervision of Hanna Staroszczyk, PhD., DSc., Associate Professor.

Bioactive peptides are currently one of the most important areas of research in the chemical sciences. Interest in these compounds arises from their potential biological properties, such as antihypertensive, antidiabetic, antioxidant, and neuroprotective activities. These peptides are produced through enzymatic or chemical protein degradation and are increasingly considered as components of functional foods and preparations with possible health-promoting significance. Consequently, an important direction of research is the search for new, alternative sources of protein raw materials.

One potential source of such raw materials is keratin, the main component of poultry feathers. This protein is characterized by a high content of sulfur-containing amino acids and considerable structural resistance. Poultry feathers are generated in large quantities as a by-product of the poultry industry and continue to pose both technological and environmental challenges. Their use for the production of bioactive peptides may provide a valuable solution, consistent with the principles of rational waste management and the concept of a circular economy.

Another important issue related to the production of peptides from proteins is the impact of subsequent chemical transformations on their biological properties. One process that may lead to changes in peptide structure is the Maillard reaction, which commonly occurs during food processing. Such modifications can significantly affect the biological activity of peptides as well as their bioavailability.

Therefore, a comprehensive characterization of keratin-derived peptides and the products of their transformations constitutes a justified and timely subject of research, of particular importance with regard to technological processes in which interactions between proteins and carbohydrates occur. The analysis of these issues allows for a better understanding of the

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impact of chemical transformations on the properties of peptides, which confirms the relevance of the undertaken research topic. Consequently, it can be concluded that the subject of the doctoral dissertation of Mr. Antoni Taraszkiewicz, M.Sc., Eng., was appropriately chosen and holds significant importance both from the perspective of fundamental research and potential practical applications.

The doctoral dissertation is based on five thematically related scientific publications, published between 2022 and 2025 in renowned, peer-reviewed English-language journals, including *Current Pharmaceutical Design, Food Chemistry, and Critical Reviews in Food Science and Nutrition*. One of the articles submitted as part of the doctoral work is currently under review. Accordingly, the doctoral documentation includes a manuscript made available in the SSRN repository, which allows for the rapid dissemination of research results at an early stage of publication. The total Impact Factor of all publications included in the dissertation amounts to 31.5, while their total score according to the MNiSW classification reaches 670 points.

It is worth emphasizing that all publications included in the doctoral dissertation were published in journals indexed in the Journal Citation Reports database, with three of them belonging to the first quartile (Q1), which attests to their high scientific quality and significance for the development of the discipline. An analysis of the Author's contribution to the individual works shows that in the first and fourth publications his contribution amounted to 50%, in the second publication 44%, in the third publication, currently under review, 15% (third author), while in the review article published in Critical Reviews in Food Science and Nutrition the Author's contribution was estimated at 70%. It should also be noted that, despite the absence of one co-author's signature on the statements attached to the dissertation, the content of the declarations included in the publications clearly confirms the leading role of Mr. Antoni Taraszkiewicz, M.Sc., Eng., in the execution of the presented research.

It is particularly worth noting that the research conducted within the doctoral dissertation was carried out as part of the project entitled "Characterization of bioactive peptides from chicken feather keratin and products of their transformations occurring during the Maillard reaction" (No. DEC-2021/41/N/NZ9/04466), funded by the National Science Centre under the Preludium 20 program in the period from January 3, 2022, to January 2, 2026, with the Doctoral Candidate serving as the principal investigator.

The Doctoral Candidate also gained experience during international research internships at the *Food Bioavailability Laboratory, Department of Food Biosciences, Teagasc Food Research Centre, Moorepark, Ireland* - the first under the "STER – Internationalisation of Doctoral Schools" program (July 31, 2023 - October 31, 2023), and the second under the Erasmus+ program (September 5, 2022 - December 19, 2022), supervised by Dr. Linda Giblin. These

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internships enabled direct collaboration with foreign research institutions and the expansion of practical skills related to the conducted research.

The doctoral dissertation was prepared entirely in English and includes a detailed descriptive section of 67 pages, providing a comprehensive presentation of the conducted research. It begins with an introduction presenting the current state of knowledge on bioactive peptides and keratins, their properties, and their significance in the context of chemical and technological processes, including the Maillard reaction. Subsequently, the objectives of the study are defined, research hypotheses are formulated, and the scope and research tasks are presented. The descriptive section also contains a detailed analysis of the results, conclusions, as well as abstracts in Polish and English, a list of abbreviations, numerous tables and figures, and an extensive bibliography comprising 134 references. Such a broad coverage of the literature allows the conducted research to be placed in the context of current scientific achievements and demonstrates the Author's thorough preparation. Following the descriptive section, the publications included in the dissertation are presented, documenting the results of the experimental research, along with a summary of the Doctoral Candidate's scientific output. The dissertation as a whole has been prepared in a clear, coherent, and logical manner, facilitating the tracking of the research process, interpretation of the results, and assessment of the Author's contribution to the development of the discipline of chemical sciences.

The aim of this doctoral dissertation was to evaluate the potential use of keratin derived from chicken feathers as a substrate for the production of bioactive peptides and Maillard reaction products, using a hybrid approach combining *in silico* analyses with *in vitro* studies. The work focuses on the biological and functional properties of these preparations, as well as their stability and activity after simulated gastrointestinal digestion, considering their potential application as functional food ingredients. The formulated hypotheses included, among others, the effectiveness of predictive methods, the possibility of obtaining peptides with desired bioactive properties through controlled reduction and enzymatic hydrolysis, and the enhancement of antioxidant activity via the Maillard reaction. The research objective was clearly defined, and the applied materials and methods are characterized by a high substantive level and raise no concerns.

The first publication within the cycle, published in the journal *Current Pharmaceutical Design*, concerned the prediction of bioactive peptides derived from chicken feather keratin and pig hair using computational protein sequence analysis with consideration of biologically active fragments. The studies demonstrated that keratin sequences from both raw materials contain numerous peptide fragments exhibiting the ability to inhibit angiotensin-converting enzyme (ACE), dipeptidyl peptidase-4 (DPP-IV), and prolyl oligopeptidase (POP). The evaluation of theoretical keratin hydrolysates using five conventional proteolytic enzymes made it possible to identify enzymes most predisposed to the generation of short peptides with

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high biological potential and low risk of toxicity. Fragment analysis showed that keratin can serve as a source of multifunctional peptides capable of modulating several physiological processes simultaneously. Based on these results, further experimental studies in the doctoral dissertation focused on chicken feather keratin due to its greater availability and its importance in the context of waste management.

The second article in the series focuses on the chemical composition and functional properties of keratin obtained from chicken feathers and its enzymatic hydrolysates. The first objective of the study was to determine the conditions under which L-cysteine (L-Cys) functions most effectively as a reducing agent. This enabled maximum keratin recovery while maintaining its water solubility, peptide bond integrity, and amino acid quality. The obtained extract was intended to serve both as a standalone protein and as a substrate for the enzymatic release of peptides with potential biological activity. In the subsequent stage, the extract was subjected to enzymatic hydrolysis using trypsin, chymotrypsin, pepsin, and subtilisin, in order to verify the predictions obtained from *in silico* analyses. The results showed that the efficiency of hydrolysis depended on the type of enzyme and the degree of peptide degradation. Low-molecular-weight hydrolysates exhibited high solubility and retained bioactive potential. Evaluation of functional properties demonstrated that enzymatic hydrolysis reduced gelling, foaming, and fat-binding capacities, while moderately stable emulsifying properties were maintained. The amino acid profile of the extract indicated a high content of cysteine, proline, and hydrophobic residues, favoring the formation of bioactive peptides, though a deficiency of certain essential amino acids was noted. The findings emphasize that careful optimization of extraction and hydrolysis conditions is crucial for obtaining high-quality keratin and its hydrolysates, which constitute a valuable source of bioactive peptides with potential applications in functional foods.

The third article analyzes the effect of extraction using L-cysteine and subsequent enzymatic hydrolysis on the chemical structure, spatial conformation, and thermal properties of keratin obtained from chicken feathers. The results showed that the extraction process led to partial loosening of the protein structure. A decrease in crystallinity and a slight reduction in α-helix content were also observed, while the integrity of the peptide chain was maintained. Enzymatic hydrolysis further modified the keratin structure, with the effect depending on the enzyme used. Subtilisin induced the most pronounced changes in secondary structure and thermal stability, whereas trypsin, chymotrypsin, and pepsin caused only minor modifications. In summary, the results confirm the effectiveness of the two-step strategy, reduction and hydrolysis, in the controlled release of peptides from keratin, while also indicating significant differences in the action of individual enzymes. The conclusions drawn are of considerable importance for planning further research on bioactive peptides and their functional properties.

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The fourth article is devoted to the evaluation of antioxidant activity and nutritional safety of keratin isolated from chicken feathers, its enzymatic hydrolysates, and products formed as a result of the Maillard reaction and simulated gastrointestinal digestion. A set of complementary antioxidant assays was applied in the study. The results showed that enzymatic hydrolysis increased the overall antioxidant activity. The highest values were observed following the use of subtilisin, with low-molecular-weight peptides contributing most significantly to the activity. Maillard reaction products obtained with glucose or xylose retained water solubility and exhibited modified radical scavenging capacity and reducing power. Selected samples were subjected to simulated gastrointestinal digestion, allowing assessment of their digestibility, functional stability, and nutritional safety under conditions approximating physiological ones. All preparations were well absorbed, and their antioxidant activity was maintained after digestion. Moreover, no cytotoxicity was observed, confirming that chicken feather keratin, its hydrolysates, and Maillard reaction products may constitute a safe source of bioactive peptides with potential applications in functional foods.

The fifth article in the series was a review focused on the role of POP, an enzyme involved in numerous physiological and pathological processes, including the development of neurodegenerative and neuropsychiatric diseases. The potential activity of peptide POP inhibitors derived from food products such as milk, meat, fish, or plants was discussed, as these compounds may exhibit cognitive-supporting and neuroprotective properties. The analysis showed that chicken feather keratin may serve as a source of such inhibitors due to its high content of proline residues and hydrophobic amino acids, and *in silico* predictions together with experimental data confirmed the presence of peptide fragments with POP-inhibitory potential.

The doctoral dissertation is concise and clear, and the few minor editorial shortcomings do not detract from its high scientific value. The innovative nature of the conducted research and its significant potential for the development of new research directions in the exact and natural sciences should be emphasized. The main achievement of the dissertation is the development of an effective strategy for transforming chicken feather keratin into functional peptides and Maillard reaction products with antioxidant activity, while maintaining chemical integrity and low toxicity of the preparations. The research results demonstrated the possibility of controlling the bioactive and functional properties of keratin through optimization of L-cysteine reduction, enzymatic hydrolysis, and the conditions of the Maillard reaction. At the same time, they confirmed the stability and activity of the obtained preparations after simulated gastrointestinal digestion, which constitutes an important step toward their application in functional foods.

I assess the doctoral dissertation very highly. It is substantively sound, coherent, and makes a significant scientific contribution to the field of the natural and exact sciences, within the

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discipline of chemical sciences. Considering that the presented results have already been published, which required obtaining positive evaluations from reviewers, I would like to pose only a few supplementary questions:

1. Was the possibility of using other enzymes or their combinations considered in order to enhance the release of bioactive peptides from keratin?
2. What nutritional or technological limitations may result from the low content of certain essential amino acids in the obtained extract?
3. Could the use of other sugars in the Maillard reaction lead to the formation of products with higher antioxidant activity while maintaining biological safety?
4. What are the prospects for scaling the described methods to an industrial level, particularly with regard to protein recovery, control of peptide quality, and minimization of production costs?

In addition to the dissertation submitted for evaluation, the scientific activity of the Doctoral Candidate also deserves recognition. He is a co-author of six publications with a total Impact Factor of 37.3, corresponding to 810 MNiSW points. Furthermore, he actively participated in the implementation of national and international projects, and his achievements have been repeatedly recognized through the awarding of scientific prizes and scholarships. In addition to his research accomplishments, the Doctoral Candidate also stands out for his teaching and organizational activities. He supervised and co-supervised diploma theses of engineering and master's students and contributed to the popularization of science through presentations at national and international conferences. Moreover, he was actively involved in the organization of scientific events. Taken together, his achievements indicate a high level of research independence, commitment, and contribution to the development of the natural and exact sciences.

In summary, the dissertation of Mr. Antoni Taraszkiewicz, M.Sc., Eng., deserves a very high evaluation due to the significant cognitive and practical contribution of the presented research. The doctoral dissertation constitutes an example of an original and carefully planned research project, making a meaningful contribution to the development of the natural and exact sciences, within the discipline of chemical sciences. The Doctoral Candidate demonstrated comprehensive theoretical knowledge, high competence in conducting independent research, as well as the ability to reliably interpret and critically analyze the obtained results. Particular recognition should be given to his well-developed analytical skills, diligence in carrying out experiments, and the ability to formulate conclusions based on the obtained scientific research results. The doctoral dissertation is characterized by a high level of clarity and a careful integration of experimental research with literature analysis. Moreover, the Doctoral

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Candidate demonstrated engagement in scientific, teaching, and organizational activities, which further emphasizes the comprehensive nature of his achievements.

The doctoral dissertation submitted for evaluation by Mr. Antoni Taraszkiewicz, M.Sc., Eng., entitled "Characterization of bioactive peptides from chicken feather keratin and products of their transformations occurring during the Maillard reaction" fully meets the statutory requirements set forth for doctoral dissertations pursuant to Article 187 of the Act of July 20, 2018 - Law on Higher Education and Science (Journal of Laws of 2023, item 742). **Therefore, I have the honor to submit to the High Council of the Discipline of Chemical Sciences at Gdańsk University of Technology a motion to admit Mr. Antoni Taraszkiewicz, M.Sc., Eng., to the subsequent stages of the doctoral proceedings.**

This dissertation is distinguished by an innovative approach to the utilization of chicken feather keratin, combining sequential reduction with L-cysteine, enzymatic hydrolysis, and the Maillard reaction in order to obtain bioactive peptides and products with high antioxidant activity. The work presents comprehensive studies of the structural, physicochemical, and biological properties of the obtained preparations, which allowed for the formulation of conclusions of considerable cognitive and practical significance. The applied solutions offer products with improved functional properties and biological safety. In addition to its scientific value, the dissertation demonstrates clear implementation potential. The developed procedures may be scaled up to industrial production, enabling efficient keratin recovery, quality control of the obtained peptides, and minimization of production costs. The Doctoral Candidate's research independence, precise experimental design, and ability to analytically interpret data confirm a high level of competence, while the **innovative and practically applicable solutions justify a recommendation for distinction of the dissertation**. Furthermore, the Doctoral Candidate's significant scientific output, confirmed by a high cumulative publication impact factor, research activity, participation in national and international projects, and teaching experience, testify to his well-developed competencies and scientific independence. Taking into account the above aspects, including the importance of the research topic, the innovativeness of the dissertation, and its practical implementation potential, **I hereby submit a motion to award distinction to this doctoral dissertation**.

Signature Not Verified
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