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Review and assessment of the doctoral dissertation
of M.Sc. Eng. Antoni Taraszkiewicz
titled:

„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, Food Technology and Biotechnology at the Faculty of Chemistry of the Gdańsk University of Technology. The supervisor of the thesis is Dr. Hab. Hanna Staroszczyk.

The dissertation is prepared in the form of a collection of published articles. The dissertation consists of four published research articles and one review article. The articles were published between 2022 and 2026. The dissertation and its annexes consist of 179 pages. It is written in English, but contains the title and summary also in Polish.

The work generally concerns the development of a method for processing chicken feathers into peptides for food applications. As part of his doctoral thesis, the PhD student combined *in silico* simulation of peptide release with experimental analysis of the composition, structure, thermal stability, antioxidant activity and nutritional safety of keratin before and after processing.

The problem of food industry waste is still relevant. Skillful management of this waste is a constant challenge for both researchers and the food industry. Chicken feathers are a by-product of poultry processing, and their appropriate management can lead to valuable products for both the food and cosmetics industries. Therefore, the challenges undertaken by a PhD student as part of the doctoral thesis are on time and capable of leading to innovative research and industrial solutions.

In the theoretical part, the author described the importance of peptides in biotechnological applications, food and pharmaceuticals. He reviewed bioactive peptides, highlighting the antioxidant, anti-inflammatory, antimicrobial, neuroprotective, antihypertensive and other properties of selected peptides.

Next, he described the methods of obtaining bioactive peptides from selected proteins, including enzymatic, microbiological, chemical hydrolysis and those in the digestive system; he clearly presented the advantages and disadvantages of each method in Table 1. Then he described the modification of bioactive peptides by the Maillard reaction. Chapter 2 is focused on keratin. It discusses the types of keratin, its physicochemical properties, and the methods and sources of its extraction. The author also discusses the mechanisms of hydrolysis and solubilization of keratin based on the latest scientific articles.

In the experimental part, the author presented the purpose of the work and the hypotheses. The aim of his study was to evaluate the suitability of chicken feather keratin as a precursor of bioactive peptides and MRPs through a hybrid *in silico-in vitro* approach. Based on scientific reports, six hypotheses were presented, which were verified later and described as part of the doctoral thesis. Research tasks were presented, the implementation of which led to the achievement of the intended goal. The stages of research work have been very interestingly presented graphically in the diagram in Figure 11.

Next, the PhD student briefly discussed the research articles and review paper included in the doctoral dissertation. All the articles constituting the doctoral dissertation are multi-authored and are numbered in the dissertation from A1 to A5.

Original articles presenting research results include:

A1. Taraszkiewicz, A., Sinkiewicz, I., Sommer, A., Dąbrowska, M., & Staroszczyk, H*. (2022). Prediction of bioactive peptides from chicken feather and pig hair keratins using *in silico* analysis based on fragmentomic approach. *Current Pharmaceutical Design*, 28(10), 841–851.

A2. Taraszkiewicz, A., Sinkiewicz, I., Sommer, A., Kusznierewicz, B., Giblin, L., & Staroszczyk, H.* (2025). Chemical composition and techno-functional properties of highpurity water-soluble keratein and its enzymatic hydrolysates. *Food Chemistry*, 472, 142641.

A3. Staroszczyk, H., Sommer, A., **Taraszkiewicz, A.**, & Michalec M. Changes in the structure and thermal properties of feather keratin induced by L-Cys extraction followed by enzymatic hydrolysis. https://doi.org/10.2139/ssrn.5382858 *(under review-published in 2026)*

A4. Taraszkiewicz, A., Sommer, A., Sinkiewicz, I., Koss-Mikołajczyk, I., Kusznierewicz, B., Giblin, L., & Staroszczyk, H. (2025). Valorising feather keratin: Antioxidant activity, cytotoxicity and mutagenicity during processing and gastrointestinal digestion. *Food Chemistry*, *493P2*, 145820.

In three of the four mentioned research articles, Antoni Taraszkiewicz is the first author but does not act as a corresponding author. The statements included in the dissertation clearly indicate the leading role of the PhD student in the published works. The percentages were estimated at 50% in articles A1 and A4, 15% in article A3, and 44% in article A2. The scientific journals listed above belong to a group of highly reputable scientific journals, where articles are subject to detailed and critical evaluation by reviewers in their respective fields. A review article with 70% participation of the PhD student was also published in a good scientific journal from the Critical Reviews series.

A5. Taraszkiewicz, A., Sinkiewicz, I., Sommer, A., & Staroszczyk, H. (2024). The biological role of prolyl oligopeptidase and the procognitive potential of its peptidic inhibitors from food proteins. *Critical Reviews in Food Science and Nutrition*, 64(19), 6567–6580.

Article A1 presents an in silico analysis of the primary structure of keratins from chicken feathers and pig hair, which constitutes the computational basis of the doctoral dissertation. Initially, entire keratin sequences were analyzed for the presence of known bioactive peptides using the BIOPEP-UWM database, determining the occurrence of bioactivity types. Potential functionalities available for release in both tested keratins were indicated. Then, both types of keratin were hydrolyzed using known proteases and the obtained hydrolysates were tested. The efficiency of hydrolysis was also compared depending on the enzyme used.

Article A2 presents the results of studies conducted only on chicken feather keratin and presents the conditions for L-Cys reduction to maximize keratin extraction efficiency while maintaining peptide bonds and amino acid quality. The target product was a high-purity keratin extract for food applications and further enzymatic release of potentially bioactive food-grade peptides. The author showed that precise optimization of each processing step is necessary for production high-quality, bioactive keratin preparations from feather waste.

Article A3 describes the research results determining the physicochemical changes caused by extraction and subsequent proteolysis, as well as the structure and thermal properties of the obtained keratin preparations. At the time of submission of the doctoral dissertation, the article was under review, but it has already been published online in Food Chemistry for 2026. It was shown in article A3 that extraction reduced the α-helix content of keratin in favor of a β-sheet structure, leading to less ordered crystal structures. These structural changes reduced the thermal stability of keratin, causing it to degrade more slowly but more gradually. The tested hydrolysates showed preserved amide structure features, but reduced α-helix and β-sheet content and crystallinity. Subtilisin induced the most profound structural and molecular changes

among the enzymes used, including loss of secondary structure, formation of carboxylate groups and production of short peptide fragments.

Article A4 describes the study of the antioxidant activity of the obtained keratin peptides. The ABTS, Folin-Ciocâlteu method and the Fe^{2+} chelating activity test were used for the investigations. To assess the safety of keratin hydrolysates, the MTT test on human enterocytes and the mutagenicity test using the Ames test were used. Representative keratin preparations from each processing step were tested using the static INFOGEST protocol to assess their digestibility and bioavailability under appropriate physiological conditions.

Article A5 is a review paper describing prolyl oligopeptidase (POP) involved in various physiological and pathological processes in the human body. In this paper, the authors cited 143 literature items. It summarizes the state of knowledge on POP and its peptide inhibitors derived from food proteins, with particular emphasis on their potential as cognitive enhancers. The author pointed out that the keratin preparations that he experimentally tested also showed a strong potential to inhibit POP, supported by the high Pro content in keratin.

To sum up, the PhD student Antoni Taraszkiewicz obtained many interesting results.

Based on the simulations performed, he showed that keratin is, among others, a source of dipeptidyl peptidase IV, angiotensin-converting enzyme and proline oligopeptidase inhibitors as well as antioxidant peptides. He showed that L-Cys keratin extraction decreases the crystallinity and α-helix content of native protein in favour of β-sheet, resulting in its slower but more gradual thermal decomposition. The isolate obtained showed high purity, good water solubility, retained backbone and favorable gelling, oil-binding and foaming. Isolate hydrolysis with trypsin, chymotrypsin and pepsin only slightly modified protein secondary structure but altered techno-functional properties in a degree of hydrolysis- and pH-dependent way. Subtilisin hydrolysate showed the most significant changes in protein structure, resulting in the best solubility, antiradical, Fe^{2+} chelating and reducing properties. The glycation products of this hydrolysate with Xyl had enhanced antioxidant activity post simulated gastrointestinal digestion. Neither processing nor digestion of keratin induced cytotoxic or mutagenic effects.

The PhD student demonstrated that he can use bioinformatics tools well and write good research and review articles. The articles included in the dissertation are multi-authored, demonstrating the PhD student's ability to cooperate in a research team.

Comments:

1. Page 32, Figure 8: Is this diagram taken from the literature or made by the author? The diagram suggests a keratin double helix with branches, resembling a DNA helix. Is this really the case? In the work of Shavandi et al. (2017), the diagram does not suggest such helices.
2. In the author's opinion, can the management of waste in the form of hooves and horns to obtain good quality keratin derivatives be more profitable than the management of chicken feathers?
3. Table 2 shows the chemical composition of keratin from chicken feathers, sheep wool and pig hair based on the article by Banasaz & Ferraro. Has the chemical composition of keratin been tested at all stages of research work?
   Why was whey protein isolate used for comparison (page 87)?
4. The author used data from "the UniProt database of protein sequences" and used data for keratins containing less than 100 amino acids. In the author's opinion, could the use of longer chains give different calculation results?
5. Can soluble keratin derivatives still be called keratins or kerateins? Please explain. In article A2, page 82 in the dissertation, one can find ”soluble derivatives called kerateins are formed (Shavandi et al., 2017; Sinkiewicz et al., 2018). On page 83, there is another name, “water-soluble keratin isolate”.
6. In article A3, the authors suggest two denaturation temperatures for keratin, the first at approximately 105 ºC, the second at 280-320 ºC. Please explain whether the loss of water during protein heating is always related to denaturation.
7. Review paper A5 concerns Prolyl oligopeptidase (POP) and is somewhat loosely related to the dissertation as a whole. Why did the author decide to review the literature regarding POP?

The minor defects and questions I have mentioned do not in any way reduce the substantive value of the work, which I rate very highly. The total achievements of Mr. Antoni Taraszkiewicz are also at a very good level. The doctoral student is a co-author of 7 publications and 11 conference reports. He completed two international research internships at the Department of Food Biosciences, Teagasc Food Research Center, Moorepark, Ireland. He received a Preludium NCN grant and participated in an international grant (EEA and Norway grants). He is also active in popularizing science and advising students on their master's and

bachelor's theses. He perfectly uses available research techniques and literature to solve research tasks.

To summarize, the PhD student successfully achieved his research goals. He obtained and characterized hydrolysates from chicken feathers and pig hair. He demonstrated that careful optimization of each processing step is necessary to produce high-quality, bioactive keratin preparations from feather waste.

The dissertation presents numerous original results of scientific and practical significance. The most important achievements of the dissertation include:

1. *In silico* analysis of chicken feather and pig hair keratin sequences to predict peptide release profiles, assess potential bioactivities and toxicity;
2. Production of MRPs by reacting the enzymatic keratin hydrolysate with D-Glc or D-Xyl under controlled thermal conditions;
3. Evaluation of antioxidant activity, nutritional safety, bioaccessibility and digestibility of selected keratin preparations from different processing stages under physiologically relevant conditions using the INFOGEST SGID protocol.

Taking into account all the research results, their originality and quality, their correct discussion as well as their potential and real application, I believe that the work meets the conditions set for doctoral dissertations by Art. 187 of the Act of July 20, 2018, Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended). I am appealing to the Discipline Council of Chemical Sciences of the Gdańsk University of Technology to admit M.Sc. Antoni Taraszkiewicz to the next stages of the doctoral process.