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REVIEW OF THE DOCTORAL DISSERTATION

MSc Eng. Havva Aktaş

Entitled **"Plant-based protein-polysaccharide systems and double emulsions as polyphenol carriers – formulation, stability and functionality"**

prepared under the supervision of **Prof. Marcin A. Kurek**

at the Warsaw University of Life Sciences

Formal and legal basis of the review

This review has been prepared following my appointment as a reviewer of the doctoral dissertation of MSc Eng. Havva Aktaş, entitled "Plant-based protein-polysaccharide systems and double emulsions as polyphenol carriers – formulation, stability and functionality," by the Discipline Council of Food Technology and Nutrition of the Warsaw University of Life Sciences, at its meeting held on 10 July 2026, pursuant to Resolution No. 78/TŻiŻ-2025/2026. Notification of the appointment was provided in the letter from the SGGW Research Support Office No. BON.5100.29.2026 of 10 July 2026.

The procedure for the award of the doctoral degree is conducted pursuant to the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended) and Resolution No. 89–2022/2023 of the Senate of the Warsaw University of Life Sciences of 26 June 2023 on the adoption of the Regulations governing procedures for the award of doctoral degrees at the Warsaw University of Life Sciences.

Pursuant to Article 187 of the Act and §§ 9 and 15 of the aforementioned Regulations, the principal purpose of this review is to assess whether the dissertation demonstrates the Doctoral Candidate's general theoretical knowledge within the discipline, confirms her ability to conduct independent scientific research, and constitutes an original solution to a scientific problem or an original solution concerning the application of the results of her own scientific research in the economic or social sphere.

General assessment and justification of the research topic

The design of delivery systems capable of protecting, stabilising, and facilitating the effective utilisation of bioactive compounds constitutes an important area of contemporary food science and technology. Polyphenolic compounds are of particular interest because, despite their widely documented biological activities, their technological utilisation may be restricted by their susceptibility to pH, temperature, light, oxygen, as well as processing and storage conditions. These limitations justify continued research into protective matrices and encapsulation technologies.

In parallel, increasing scientific attention is being directed towards plant proteins as structure-forming, interfacially active, emulsifying, and carrier materials. Of particular relevance is the exploration of unconventional protein sources, including agri-food processing by-products. Such materials may provide functional biopolymers with properties distinct from those of more conventional plant-protein materials, particularly soy-based reference systems.

The doctoral dissertation of MSc Eng. Havva Aktaş fits very well within these current research directions. Its central focus is the utilisation of plant protein–polysaccharide systems and double emulsions as carriers of polyphenols, with particular emphasis on anthocyanins and, in one part of the study, green tea catechins.

A particularly valuable aspect of the work is that the carrier material is not approached merely as a passive physical barrier. Instead, the Doctoral Candidate attempts to relate protein origin and structure, physicochemical and interfacial properties, environmental conditions, and carrier architecture to the final functionality of the delivery system. This approach advances the research beyond purely empirical optimisation of individual formulations and towards the identification of structure–property–function relationships.

The selection of protein sources also deserves recognition. The study includes proteins obtained from canola seed cake, black caraway seed cake, wheat bran, sunflower meal, mustard meal, and evening primrose meal. Their comparison with more conventional plant proteins enabled the evaluation of materials with different botanical origins, processing histories, and structural properties.

I therefore consider the subject of the dissertation to be highly relevant, scientifically justified, and important to the discipline of Food Technology and Nutrition, both from a fundamental and a prospective technological perspective.

Formal assessment of the dissertation and publication series

The doctoral dissertation is based on a collection of six thematically related scientific publications:

1. **Aktaş H., Custodio-Mendoza J., Szpicer A., Pokorski P., Samborska K., Kurek M.A. (2024).** *Polysaccharide-potato protein coacervates for enhanced anthocyanin bioavailability and stability.* International Journal of Biological Macromolecules, 282, 136829.

2. **Aktaş H., Custodio-Mendoza J., Moczkowska-Wyrwisz M., Szpicer A., Kurek M.A. (2024).** *The role of canola, black caraway, and wheat bran protein isolates in anthocyanin microencapsulation via double emulsions.* Industrial Crops and Products, 222, 119644.

3. **Aktaş H., Custodio-Mendoza J.A., Samborska K., Kurek M.A. (2025).** *Functional and structural performance of canola, black caraway, and soy proteins in anthocyanin microencapsulation.* Food and Bioprocess Technology, 18(12), 10317–10334.

4. **Aktaş H., Szpicer A., Strojny-Cieślak B., Borucki W., Schweiggert-Weisz U., Kurek M.A. (2025).** *Molecular Interplay Between Plant Proteins and Polyphenols: pH as a Switch for Structural and Functional Assembly.* Foods, 14(23), 3991.

5. **Aktaş H., Napiórkowska A., Szpicer A., Custodio-Mendoza J.A., Paraskevopoulou A., Pavlidou E., Kurek M.A. (2024).** *Microencapsulation of green tea polyphenols: Utilizing oat oil and starch-based double emulsions for improved delivery.* International Journal of Biological Macromolecules, 274, 133295.

6. **Aktaş H., Kurek M.A. (2024).** *Deep eutectic solvents for the extraction of polyphenols from food plants.* Food Chemistry, 444, 138629.

According to the data presented in the dissertation, the combined Impact Factor of these publications is 43.9, while their total score in the Polish ministerial journal ranking is 800 points. I consider these bibliometric indicators supplementary information; the principal basis for the present assessment remains the scientific merit, originality, methodological quality, and individual contribution of the Doctoral Candidate.

The first five publications are experimental papers. Publication P6 is a review article addressing the application of deep eutectic solvents to polyphenol extraction. The Doctoral Candidate appropriately identifies P6 in the methodological section as a non-experimental component of the dissertation serving primarily a theoretical and integrative role.

A detailed examination of the co-authorship statements demonstrates the Doctoral Candidate's substantial and creative contribution to all publications forming the dissertation. Her involvement consistently included conceptualisation, methodological development, experimental work, formal analysis, validation, visualisation, and manuscript preparation. She was the first author of all six publications. This level of involvement provides strong evidence of her scientific independence and her ability to participate actively in all major stages of the research process.

The schematic overview of the research stages presented on page 15 is particularly helpful in illustrating the progression of the project from the selection and characterisation of protein and polyphenol sources, through double-emulsion and coacervation systems, to pH-dependent studies of protein–anthocyanin complexes.

Scientific assessment of the dissertation

Research objectives and hypotheses

The principal objective of the dissertation was to elucidate the interactions between anthocyanins and selected plant proteins and polysaccharides and to evaluate the resulting complexes, double emulsions, and complex coacervates as plant-based carrier systems for anthocyanins.

The specific objectives included the characterisation of structural and functional properties of proteins derived from agri-food by-products, investigation of pH-dependent protein–anthocyanin complex formation, development of double-emulsion systems, and design of potato protein–polysaccharide coacervates.

Five research hypotheses, H1–H5, were formulated.

The hypotheses are predominantly centred on anthocyanin-containing systems, whereas P5 extends the experimental scope to green tea catechins and P6 provides a broader theoretical perspective on polyphenol extraction. I regard these elements as complementary extensions of the central research concept rather than as a limitation of the thematic coherence of the dissertation.

Experimental and analytical methodology

The methodological breadth of the dissertation constitutes one of its major strengths. The experimental programme integrates complementary structural, spectroscopic, colloidal, interfacial, rheological, thermal, microscopic, chromatographic, and functional approaches, enabling the investigated systems to be characterised at multiple levels — from protein structure and intermolecular interactions to the properties of emulsions, coacervates, and microcapsules.

The analytical strategy included, among others, electrophoretic and spectroscopic characterisation of proteins, assessment of surface and colloidal properties, rheological and thermal analyses, microscopic observations, chromatographic determination of polyphenolic compounds, and evaluation of encapsulation efficiency, retention, and antioxidant activity. Taken together, these methods demonstrate the Doctoral Candidate's strong experimental competence and her ability to integrate approaches originating from food science, protein chemistry, and colloid science.

Particularly noteworthy is the complementary methodology employed in P4, which provides convincing evidence of pH-dependent structural and colloidal changes in protein–polyphenol systems. The results support the involvement of electrostatic, hydrophobic, and hydrogen-bonding interactions; however, the applied techniques do not allow the relative contribution of these individual interaction mechanisms to be resolved unequivocally.

This limitation is important when interpreting pH-dependent behaviour, since pH simultaneously affects protein ionisation, conformation, solubility, and aggregation, while also influencing anthocyanin speciation and stability. Consequently, the mechanistic interpretations proposed in the dissertation are scientifically plausible and well supported by complementary

evidence but should be regarded as interpretations rather than as direct identification of individual intermolecular forces.

Plant protein characterization: from source to functionality

The results demonstrate pronounced differences among the investigated protein isolates in terms of protein purity, molecular weight distribution, secondary structure organisation, solubility, surface hydrophobicity, and charge behaviour.

In P4, sunflower meal protein showed the highest protein content among the proteins examined in that study, followed by mustard and evening primrose proteins. SDS-PAGE profiles confirmed substantial variation in the relative contributions of different protein fractions and molecular weight regions.

FT-IR analysis further demonstrated differences in secondary structure and in the susceptibility of these structures to pH-induced changes.

Importantly, the results do not identify a single protein parameter that can universally predict carrier functionality.

Instead, they indicate that favourable carrier performance results from a particular combination of solubility, conformational flexibility, hydrophobicity, interfacial properties, and formulation environment.

I consider this to be one of the most important and scientifically valuable conclusions of the dissertation.

Double emulsions stabilised by proteins derived from agri-food by-products

Publications P2 and P3 provide valuable information concerning the use of alternative plant proteins in $W_1/O/W_2$ double-emulsion systems.

P2 evaluated proteins derived from canola, black caraway, and wheat bran in comparison with soy protein. Anthocyanin inclusion efficiency was high for several formulations, whereas emulsion stability and droplet-size distribution were considerably more variable.

This finding is technologically significant because it demonstrates that high initial encapsulation efficiency should not be regarded as synonymous with high physical stability of the carrier system.

In P3, following extension of the process to a drying stage, particularly favourable results were obtained for the formulation containing 10% black caraway protein. In the BC10 formulation, retention of Dp-3-Glu reached 90.90%, while retention of Cy-3-Rut and Cy-3-Glu also exceeded 85%. The system also displayed favourable antioxidant and structural properties.

I consider it particularly appropriate that the authors themselves adopt a cautious interpretation in the conclusion of P3. They explicitly acknowledge that the experiments were performed under controlled laboratory conditions, that stability was assessed over a relatively short period, and that future work should include long-term storage, real food matrices, and evaluation of behaviour during digestion.

This recognition of the boundaries of the experimental evidence is scientifically appropriate.

Protein–polysaccharide coacervates

Publication P1 employed potato protein in combination with gum arabic, guar gum, inulin, pectin, and soluble fibre, while a soy protein–gum arabic system was used as a reference formulation.

The type of polysaccharide strongly affected morphology, moisture content, hygroscopicity, solubility, antioxidant activity, and microencapsulation efficiency. Microencapsulation efficiency ranged from 64.00 to 81.20%, with the highest value obtained for the potato protein–gum arabic formulation.

The findings clearly demonstrate that carrier performance depends not only on the protein itself, but also on the architecture of the complete protein–polysaccharide system.

The comparison between the mechanisms of complex coacervation and double-emulsion stabilisation is also valuable. The dissertation interprets coacervate performance primarily in terms of polymer-rich phase separation and volumetric entrapment, whereas double-emulsion behaviour is discussed mainly in relation to interfacial stabilisation by proteins.

This part of the dissertation, however, raises an issue that requires clarification regarding hypothesis H4. In P1, the reference system was soy protein–gum arabic, which is also a two-component protein–polysaccharide system. I did not identify a direct experiment in which a potato protein–polysaccharide coacervate was compared, under otherwise identical conditions, with the corresponding system containing only potato protein.

Accordingly, the comparative component of H4 — namely, the proposed higher loading capacity and improved stability relative to corresponding single-protein systems — was not tested in a directly matched formulation under otherwise identical conditions.

pH as a determinant of protein–polyphenol complex behaviour

I consider P4 to be one of the most scientifically interesting elements of the dissertation.

The study investigated the influence of pH on mustard, evening primrose, and sunflower proteins and their systems in the presence of red-cabbage-derived polyphenols. Solubility, hydrophobicity, free sulfhydryl groups, secondary structure, fluorescence, ζ-potential, particle size, functional properties, thermal behaviour, and microstructure were assessed.

The results clearly confirm that pH is one of the major regulators of the behaviour of these systems.

Near the isoelectric region, decreased solubility and enhanced aggregation were observed, while changes in pH altered exposure of hydrophobic regions, secondary structure, and interfacial behaviour.

The Doctoral Candidate interprets acidic conditions as favouring substantial electrostatic contributions and the formation of compact complexes, while neutral and alkaline conditions

are associated with greater conformational flexibility and hydrophobic interactions, but also with an increased risk of aggregation.

The overall direction of this interpretation is reasonable; however, caution should be exercised when using terms such as "mechanism" or "dictates". The analytical techniques employed provide a strong body of indirect evidence for structural changes and interaction patterns, but a complete thermodynamic characterisation and determination of the relative contributions of specific intermolecular forces would require additional approaches.

Importantly, P4 does not provide detailed compositional profiling of the polyphenol fraction and does not include assessments of digestion, bioaccessibility, or bioavailability. These limitations should be considered when interpreting the proposed interaction mechanisms.

Green tea polyphenols: extending the approach beyond anthocyanins

Publication P5 broadens the research towards green tea polyphenols and systems based on starch and oat oil.

The study assessed encapsulation efficiency, polyphenol retention, antioxidant activity, powder characteristics, thermal and structural properties, and individual catechins.

The reported encapsulation efficiency ranged from 19.16 to 32.80%, while polyphenol retention ranged from approximately 14.96 to 21.16%. These values are considerably lower than those observed in the best-performing anthocyanin systems.

I do not regard this as a weakness of the work. On the contrary, it provides an important argument against overgeneralisation: these results further illustrate that carrier performance is both compound- and matrix-dependent under the conditions investigated.

At these levels of encapsulation, however, I would avoid describing all P5 formulations as systems displaying high encapsulation efficiency without qualification. It is more accurate to conclude that the study demonstrated carrier-material-dependent behaviour and partial protection and retention of catechins.

Role of Publication P6

Publication P6 provides a valuable theoretical extension of the dissertation by reviewing deep eutectic solvents as alternative extraction media for polyphenols. Its role is primarily integrative rather than experimental. Consequently, conclusions linking extraction strategy directly with subsequent carrier performance should be regarded as literature-based propositions rather than relationships experimentally verified within P1–P5. This does not diminish the value of P6 as a component broadening the conceptual framework of the dissertation.

Assessment of the verification of the research hypotheses

Overall, the experimental results provide strong support for the central research concept and for the majority of the hypotheses formulated in the dissertation. The strongest direct evidence concerns the effects of protein source, carrier architecture, and pH on the structural and functional behaviour of the investigated systems. Some individual formulations of the

hypotheses are broader than the experimental comparisons performed, particularly where they refer to direct improvements relative to non-encapsulated or single-protein reference systems. These issues concern the precision of interpretation rather than the validity or scientific value of the experimental work.

Terminology and limits of interpretation

My principal terminological comment concerns the use of the terms bioavailability, bioaccessibility, and controlled delivery.

The dissertation experimentally demonstrates encapsulation, retention, physicochemical stability, maintenance of antioxidant activity, and specific functional characteristics of the developed delivery systems.

The dissertation does not, however, include digestion or bioaccessibility studies, an intestinal transport model, or in vivo experiments sufficient to establish actual systemic bioavailability. Importantly, the authors of P2 themselves identify bioavailability as an area for future research.

For this reason, statements referring to enhanced bioavailability – including the wording used in the title of P1 – extend beyond what was directly measured experimentally. It would be more precise to refer to enhanced stability and retention, as well as to the potential of these systems to improve future bioaccessibility or bioavailability.

This is primarily an issue of scientific terminology and interpretation and does not alter my positive assessment of the developed carrier systems.

A similar degree of precision should be maintained when using the term sustainability. The use of agri-food by-products as protein sources is undoubtedly valuable in terms of raw-material valorisation and is consistent with circular-economy concepts. However, the dissertation does not include life-cycle assessment, energy balances, or environmental comparison of the complete extraction, isolation, emulsification, and drying processes.

The work, therefore, demonstrates a sustainability-oriented raw-material strategy, rather than an experimentally established environmental advantage of the complete technology.

Editorial assessment and presentation

The synthetic part of the dissertation is generally clear and well organised. Some passages would benefit from more rigorous academic wording, particularly where expressions such as "dictates" or "fully confirms" imply a stronger level of evidence than that directly provided by the experiments. Minor inconsistencies in terminology and descriptions of selected materials are secondary and do not affect the scientific assessment of the dissertation.

Questions for the Doctoral Candidate

In connection with the above assessment, I would like to address the following questions to the Doctoral Candidate:

1. The dissertation uses the terms *stability*, *retention*, *bioaccessibility*, and *bioavailability*. How does the Doctoral Candidate distinguish these concepts, and which of them were directly assessed experimentally within the dissertation?

2. P4 provides complementary evidence for pH-dependent structural and colloidal changes in protein–polyphenol systems. Which experimental observations does the Doctoral Candidate consider most diagnostic of the proposed electrostatic, hydrophobic, and hydrogen-bonding contributions? What additional experimental approach could be used to distinguish more directly between these interaction mechanisms and to separate pH-induced changes in the protein from changes in anthocyanin speciation?

3. Which of the developed systems does the Doctoral Candidate currently consider the most promising for incorporation into an actual food product, and why? Which three of the following validation stages — long-term storage, digestion, bioaccessibility, testing in a food matrix, sensory evaluation, and scale-up — would she consider most critical before potential technological implementation?

These questions are intended to stimulate scientific discussion and provide the Doctoral Candidate with an opportunity to demonstrate her ability to critically interpret the results and consider their broader scientific and practical implications.

Overall assessment

Following a detailed assessment of the dissertation and the publications forming its basis, I conclude that MSc Eng. Havva Aktaş has completed an ambitious, coherent, and original research programme addressing the development and characterisation of plant-based protein–polysaccharide systems and double emulsions as carriers of polyphenolic compounds.

The dissertation demonstrates substantial methodological breadth, a strong experimental basis, and the ability to integrate structural, physicochemical, colloidal, and functional data across different carrier architectures. Particularly valuable are the systematic comparisons of alternative plant proteins, the identification of pH as a major determinant of protein–polyphenol system behaviour, and the demonstration that carrier performance depends on the combined effects of protein source, formulation conditions, and carrier architecture.

The comments raised in this review primarily concern terminological precision, the limits of mechanistic interpretation, and the extent to which some hypotheses were tested through directly matched experimental comparisons. These reservations do not undermine the reliability of the principal findings or the scientific value of the dissertation.

The scope of the experimental work, the methodological quality, the scientific coherence of the publication series, and the documented contribution of the Doctoral Candidate demonstrate her general theoretical knowledge and her ability to conduct independent scientific research.

Final conclusion

Having examined the doctoral dissertation of MSc Eng. Havva Aktaş, entitled "Plant-based protein-polysaccharide systems and double emulsions as polyphenol carriers – formulation,

stability and functionality", the publications forming its basis, and the co-authorship statements, I conclude that the dissertation demonstrates the Doctoral Candidate's general theoretical knowledge in the discipline of Food Technology and Nutrition, confirms her ability to conduct independent scientific research, and constitutes an original solution to a scientific problem.

The dissertation therefore fulfils the requirements laid down in Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science and § 9 of the Regulations governing procedures for the award of doctoral degrees at the Warsaw University of Life Sciences to persons who have completed education at a doctoral school.

I therefore request that the Discipline Council of Food Technology and Nutrition of the Warsaw University of Life Sciences admit MSc Eng. Havva Aktaş to the public defence of her doctoral dissertation.

At the same time, in view of the particularly high scientific standard of the dissertation and the above-average quality of the doctoral achievement, I respectfully submit to the Honourable Discipline Council of Food Technology and Nutrition of the Warsaw University of Life Sciences a motion for the doctoral dissertation under review to be awarded distinction, in accordance with the procedure applicable at the Warsaw University of Life Sciences.

Sabina Lachowicz-Wiśniewska, PhD, DSc
Professor at the University of Kalisz

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Kalisz, 28 August 2026

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Justification for the Motion to Distinguish the Doctoral Dissertation of MSc Eng. Havva Aktaş

I assess the doctoral dissertation of MSc Eng. Havva Aktaş very highly and consider it to represent an above-average scientific achievement that merits distinction.

My recommendation for distinction is based first and foremost on the originality of the research concept. The dissertation does not merely optimise an individual carrier formulation but attempts to establish relationships between the molecular and physicochemical properties of proteins and the performance of different carrier architectures.

The second important argument is the scope and complementarity of the experimental methodology. The combination of structural, spectroscopic, colloidal, interfacial, thermal, microscopic, chromatographic, and functional analyses enabled the investigated materials to be evaluated across several organisational levels.

Third, the dissertation makes innovative use of unconventional plant proteins derived from agri-food processing by-products and demonstrates that, under appropriate conditions, selected proteins may match or outperform conventional reference proteins in encapsulation-related parameters.

I consider the findings concerning black caraway protein, for which very high retention of selected anthocyanins was achieved in one formulation, particularly noteworthy. The systematic investigation of pH as a regulator of protein conformation and functionality is another scientifically strong component of the work.

Fourth, the Doctoral Candidate's scientific independence is well documented. She is the first author of all six publications included in the dissertation, and the co-authorship statements confirm her leading involvement in conceptualisation, methodology, investigation, data analysis, and manuscript preparation.

Fifth, the dissertation has resulted in a high-quality publication record. The experimental results have been published in recognised international journals, with the review article appearing in *Food Chemistry*. Bibliometric indices alone do not form the basis of my recommendation for distinction, but the publication record confirms that the major findings have successfully undergone independent international peer review.

Finally, the dissertation combines fundamental scientific value with clear application potential. It does not yet constitute full industrial or biological validation of the carrier systems, but it provides a strong structure–property–function basis for their further development and testing in real food matrices.

The critical comments raised in this review relate mainly to the boundaries of interpretation and to the wording of certain conclusions. They do not undermine the quality of the experimental evidence, the originality of the scientific problem addressed, or the above-average quality of the doctoral achievement.

In view of the above, I respectfully request that the Honourable Discipline Council of Food Technology and Nutrition of the Warsaw University of Life Sciences award distinction to the doctoral dissertation entitled 'Plant-based protein-polysaccharide systems and double emulsions as polyphenol carriers – formulation, stability and functionality', in accordance with the procedure applicable at the Warsaw University of Life Sciences.

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