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Review

Candidate: MSc Marcellus Arnold

Title: Innovative impregnation techniques and process optimization for shaping the physicochemical, functional, enzymatic, and sensory properties of freeze-dried apple snacks

Institution: Poznań University of Life Sciences, Faculty of Food Science and Nutrition, Department of Gastronomy Science and Functional Foods

Scientific supervision::Prof. dr hab. Anna Gramza-Michałowska

The field of the doctoral dissertation is agricultural science, specifically focused on food technology and nutrition.

1. Description of Ph.D. candidate

Marcellus Arnold completed his Master's degree at the Poznań University of Life Sciences in 2021 at the Faculty of Food Sciences, after which he continued his doctoral studies at the same institution. This dissertation is the first major scientific work presenting the outcomes of his PhD research. During his doctoral studies, he did not undertake additional employment, while earlier, during his Master's studies, he worked at Milano Sp. z o.o. S.K.K. Throughout the course of his PhD programme, he completed several research visits and internships that contributed to the development of his scientific skills. These included placements at

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Wrocław University, Suranaree University of Technology, the University of Life Sciences in Lublin, and the University of Bologna in Cesena. He also participated in Erasmus+ traineeships at the Free University of Bozen-Bolzano and the Czech University of Life Sciences.

He is the author or co-author of the five scientific articles (A1–A5) that form the core of this dissertation. Across these works, he consistently contributed to both the conceptual and practical dimensions of the research. He was directly involved in the conceptualization of A1, A2, A3, and A5, and co-developed the study framework in A4. In the original research articles (A3–A5), he played a leading role in methodological design and experimental implementation, conducting impregnation experiments, performing antioxidant and enzymatic assays, and collecting and processing analytical data. His contributions also included advanced statistical modelling (RSM, PCA, and formal analysis) as well as full engagement in manuscript development, from preparing initial drafts to revising subsequent versions.

In addition to the dissertation papers, he is the co-author of 15 other scientific publications. He has served as principal investigator in three research grants, the most recent of which is still ongoing. He has participated in three scientific conferences and has received seven awards, including the Award from the Polish Ministry of Higher Education in 2025, which recognizes outstanding scientific achievement.

Taken together, these accomplishments demonstrate his scientific independence, methodological competence, and strong research potential, fully aligning with the expectations for doctoral-level work.

2. General overview of the research, taking into account the significance of the research topic

The work presented in this PhD dissertation is based on a compilation of articles. Five articles are included and together constitute the complete body of the dissertation. The doctoral dissertation by MSc Marcellus Arnold presents a comprehensive and well-structured study on the development of functional freeze-dried apple snacks enriched with bioactive compounds from sea buckthorn and calcium, using atmospheric, vacuum,

and ultrasound-assisted impregnation techniques. The work demonstrates considerable analytical rigor through the application of a wide range of modern instrumental and statistical methods. These methodological approaches provide valuable insights into the optimization of impregnation conditions, the behavior of bioactive compounds and enzymatic systems, and the storage stability of fortified products. Taken together, the findings constitute a meaningful contribution to the field of food technology and support the development of nutrient-enriched, shelf-stable snacks with promising commercial potential.

Situated within the growing scientific and industrial interest in functional foods, the dissertation effectively addresses the need for innovative nutrient-fortified products and highlights the suitability of fruit matrices as carriers for bioactive substances. The research integrates technological processing, chemical and antioxidant analyses, enzymatic studies, and sensory evaluation into a coherent and interdisciplinary framework. This holistic approach enables a comprehensive understanding of how technological operations impact both the nutritional and quality attributes of the final product.

The topic is well chosen and convincingly justified. The dissertation highlights calcium deficiency and osteoporosis as significant global nutritional challenges, situating the research within a broader public health context informed by epidemiological evidence. Furthermore, it positions apples as a sustainable, widely consumed raw material with notable technological advantages for impregnation processes. The selection of Gala apples and sea buckthorn juice aligns with local resource availability and current sustainability trends while enhancing the practical relevance of the work.

A key strength of the dissertation lies in its interdisciplinary nature. The author adeptly connects principles from food chemistry, process engineering, antioxidant analysis, and sensory science, resulting in a broad and integrated scientific perspective. Importantly, the work also carries clear application potential, offering realistic pathways for the industrial implementation of the developed functional snack.

The dissertation is built upon five peer-reviewed scientific publications:

- A1 – Food and Bioprocess Technology (IF 5.8): a review of apple composition and phenolic extraction methods;

- A2 – Comprehensive Reviews in Food Science and Food Safety (IF 14.8): a comprehensive review on enzymatic browning in apples and its inhibition;
- A3 – Molecules (IF 4.6): Stage I — optimization of impregnation conditions with sea buckthorn juice and calcium lactate;
- A4 – Polish Journal of Food and Nutrition Sciences (IF 2.3): Stage II — atmospheric, vacuum, and ultrasound-assisted impregnation;
- A5 – Food and Bioprocess Technology (IF 5.8): Stage III — storage stability of bioactive-enriched freeze-dried apples.

These publications, with a total Impact Factor of 33.3 and 640 Ministry of Higher Education points, demonstrate significant scientific output and a well-structured research program. The candidate's contribution to each article is considerable, including conceptual design, laboratory work, statistical analysis (RSM, PCA), result interpretation, and manuscript preparation.

Across the five publications included in the dissertation, MSc Marcellus Arnold played a central and consistently substantive role in both conceptual and practical aspects of the research. His contributions span the formulation of research ideas through to the execution of experiments, data processing, and manuscript preparation.

A1 – Food and Bioprocess Technology (Review) - M.Sc. Arnold contributed to conceptualization, performed data curation, prepared the visualizations, and authored the original draft, followed by review and editing of the manuscript.

A2 – CRFSFS (Review) - His contribution was even broader, including conceptualization, data curation, investigation, methodology development, visualization, writing the original draft, and revising the manuscript.

A3 – Molecules (Stage I research) - In this original research article, his involvement was extensive and directly linked to the core of the scientific work. He contributed to conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, writing the original draft, and writing—review and editing. These contributions confirm his leading role in designing and executing Stage I of the project.

A4 – Polish Journal of Food and Nutrition Sciences (Stage II research) - Based on the list from the declaration, the candidate is listed as the first author, which indicates that he

undertook the essential responsibilities typical for first authorship, such as designing the study structure, performing key elements of the laboratory investigations, and preparing and revising the manuscript. His involvement is confirmed in the author's declaration included in the dissertation.

A5 – Food and Bioprocess Technology (Stage III research) - Here, PhD candidate Arnold's contribution mirrors the structure in A3 and once again demonstrates his leading role. He was responsible for conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, writing the original draft, and writing—review and editing. These tasks encompass the full experimental and analytical workflow of the storage study.

The scientific project was supported by multiple competitive funding sources, including:

1. National Science Centre (Preludium) — 2023/49/N/NZ9/00862,
2. Ministry of Education and Science — SKN/SP/534738/2022,
3. Regional Initiative Excellence — 005/RID/2018/19,
4. Young Scientist Grants (2022, 2023) — 506.751.03.00,
5. Statutory funds of the Department of Gastronomy Sciences and Functional Foods — 506.751.03.00.

This level of financial support reflects the relevance and quality of the research.

3. Description of the work (Relationship between the dissertation and publications A1–A5)

The research conducted by MSc Marcellus Arnold followed a clear and logically structured sequence, progressing from an extensive literature review to experimental design, optimization, comparative evaluation of technological processes, and finally, the assessment of storage stability. The doctoral candidate's work began with a thorough analysis of existing knowledge, which enabled him to define research gaps and formulate the scientific rationale for the project. This conceptual groundwork was developed through two comprehensive review articles (A1 and A2), in which he synthesized data on

apple composition, phenolic extraction methods, enzymatic browning mechanisms, and inhibition strategies. These reviews provided the necessary context for selecting sea buckthorn as a fortifying agent and for designing experiments that focused on mass transfer, bioactive retention, and enzymatic behavior.

Building on this foundation, the candidate designed and executed the first experimental stage (A3), which aimed to optimize impregnation conditions using sea buckthorn juice and calcium lactate. He conducted laboratory experiments to evaluate the influence of temperature, time, and solution composition on the uptake of bioactive compounds and calcium, as well as antioxidant capacity and enzymatic activity. Through response surface methodology, he established optimal process parameters and identified the key variables that control the migration of functional compounds. The results demonstrated that impregnation efficiency could be significantly improved by adjusting solution concentration and processing temperature, confirming the technological potential of the approach. At this stage, the author decided to discontinue using inulin in further experiments. The preliminary trials showed that the inulin-to-juice ratio remained below the threshold required to achieve meaningful technological or functional effects; therefore, the formulation was simplified to a 0:100 inulin-to-SB juice ratio.

In the second stage (A4), the doctoral candidate expanded his investigations by comparing atmospheric impregnation with vacuum and ultrasound-assisted techniques. He implemented these methods experimentally, analyzed their impact on the chemical and enzymatic characteristics of the fortified apples, and used multivariate statistical tools to interpret the results. This stage demonstrated that both vacuum and ultrasound enhanced mass transfer and antioxidant retention more effectively than atmospheric methods. Ultrasound, in particular, led to pronounced improvements in compound uptake, supporting the conclusion that it offers a promising alternative for enhancing the fortification of fruit matrices.

The third stage (A5) focused on the storage stability of the enriched freeze-dried apples over a three-month period. The candidate performed functional, enzymatic, colour, and sensory assessments to determine how the fortified products changed over time. The results indicated predictable declines in total phenolic content and antioxidant activity; however, samples enriched with sea buckthorn consistently maintained superior

functional properties compared to controls. The study also documented the behavior of browning-related enzymes during storage and confirmed that impregnation reduced enzymatic activity in several variants, thereby contributing to improved color stability. His findings demonstrated strong internal consistency: methods that proved efficient in Stage I also yielded positive results in Stages II and III. Overall, the research confirmed that the combination of sea buckthorn juice and calcium lactate is effective for fortifying freeze-dried apples. It was found that vacuum and ultrasound-assisted impregnation are particularly promising from both technological and nutritional perspectives.

The results presented in the dissertation are extensive and coherent, showing a solid understanding of food chemistry and processing mechanisms. Key observations include the high effectiveness of vacuum and ultrasound-assisted impregnation, which significantly increased antioxidant activity and calcium content compared to atmospheric impregnation. A notable nutritional enhancement was achieved with the addition of calcium lactate (CaL), where a 4% CaL formulation enabled the final products to contain over 2800 mg of calcium per 100 g, providing a significant dietary benefit.

The impregnation process also caused characteristic shifts in the phenolic and carotenoid profiles, favoring isorhamnetin derivatives and carotenoids typical of sea buckthorn. Furthermore, a reduction in the activity of polyphenol oxidase (PPO) and peroxidase (POD) was observed in several variants. The author effectively links these changes to protective antioxidant effects and structural alterations resulting from the technological treatments. As expected, there was a gradual decrease in phenolic content and antioxidant activity during storage. However, the impregnated samples consistently maintained higher functional quality compared to the controls throughout the entire three-month period.

The author demonstrates a strong ability to critically interpret findings, addressing variability between batches of raw materials and the degradation pathways of bioactive compounds. This analytical awareness enhances the credibility of the conclusions.

In light of these results, the research hypotheses formulated in the dissertation were appropriately tested and can be considered confirmed. Hypotheses H1a and H1b proposed that both process conditions and solution composition would influence the physicochemical, functional, enzymatic, and sensory characteristics of the fortified

apples. These hypotheses were supported by clear trends across all stages of the experimental work.

Hypothesis H2 predicted that vacuum and ultrasound-assisted impregnation would enhance mass transfer and improve fortification efficiency. This hypothesis was strongly validated by the significantly higher calcium uptake and antioxidant retention observed in these variants.

Finally, H3 assumed that storage would affect functional properties, yet allow enriched products to retain their quality advantage over controls. This hypothesis was confirmed by the stability data collected over the three-month study.

In summary, the experimental outcomes align directly with the research hypotheses, and the evidence presented in the dissertation supports the conclusion that all hypotheses were properly formulated and empirically verified.

4. Questions and (Relationship between the dissertation and publications A1–A5)

While the dissertation is scientifically strong and presents a well-developed research concept, several aspects would benefit from clarification to further strengthen the transparency and completeness of the work. These comments are intended as constructive suggestions that may help refine the interpretation of the results.

1. You noted in the review of the literature that Gala apples are a popular and accessible variety, but no other specific properties are detailed. Additionally, a brief discussion of their technological suitability (e.g., texture, enzymatic activity) would enhance the methodological rationale. **What factors influenced the choice of the Gala cultivar for impregnation studies?**
2. In publications A3 and A5, apples were frozen before processing; however, the dissertation does not explain the reason for this storage choice. Since freezing can affect tissue structure and mass transfer, a brief explanation would help clarify the methodological context. **Please clarify the purpose of pre-freezing the apples**

before OD (parts I and III of the research) and its potential effects on the impregnation process?

3. Since freezing may modify the apple matrix, including such a control could have facilitated more accurate comparisons. Please explain: **How might a frozen, non-impregnated control have contributed to the interpretation of structural and enzymatic effects?**
4. The dissertation mentions initial consideration of inulin but does not fully explain why it was ultimately excluded. **Could you clarify which factors led to the decision to proceed without inulin in subsequent stages? Why did you not decrease the ratio of inulin below 15:85 and check it also?**
5. **Why did you state** in the conclusions of part 1 that "Further studies to balance the flavor should be considered"? What data supports this conclusion?
6. The storage study included seven sensory panelists, which is typical for exploratory research, but this limits the statistical scope. **How does the author view the reliability of sensory trends given the panel size?**
7. Although the dissertation compares atmospheric, vacuum, and ultrasound-assisted methods, a concluding statement identifying the most promising technique would help contextualize the practical relevance of the findings. **Which impregnation method appears to be the most appropriate considering efficiency, product quality, and technological feasibility?**

A. Clarification points

a. In the results section, the PhD candidate noted pre-freezing at stages 1 and 3, yet no data regarding this process were included in the text. Only A5 specifies freezing at –40 °C, while similar details are lacking in A3. It would be beneficial to clarify whether the raw materials at different stages were treated in a similar manner.

b. For Articles A3 and A5, supplementary materials are mentioned in the articles, but neither of the PhD candidates included them in the dissertation.

5. Final Conclusions

The doctoral dissertation by Marcellus Arnold, titled "Innovative impregnation techniques and process optimisation for shaping the physicochemical, functional, enzymatic, and sensory properties of freeze-dried apple snacks," constitutes an independent, well-defined, and methodologically sound solution to the research problem addressed. All research objectives and tasks outlined in the thesis have been fully achieved. The experimental results and the conclusions drawn from them are coherent, scientifically justified, and hold significant potential for practical application.

The author has demonstrated the ability to formulate a research concept aligned with contemporary trends in food technology, conduct complex experimental work, critically interpret findings, and engage in scientific discussions. His work reflects independence, analytical competence, and a mature understanding of the technological and biochemical mechanisms underlying the studied processes.

In my opinion, and in accordance with the applicable regulations, Marcellus Arnold's doctoral dissertation meets the requirements for the award of the degree of Doctor of Science. I therefore submit to the Scientific Council of the Food Technology and Nutrition Discipline at the Poznań University of Life Sciences a formal motion to accept the dissertation and to admit the author to public defense.

Furthermore, considering the scope and complexity of the research, the doctoral candidate's substantial personal contribution to its execution, and the high scientific quality and dissemination of the results in reputable international journals with significant impact factors, I formally request that the Scientific Council award a distinction to Marcellus Arnold's doctoral thesis.

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Nomination for Distinction

The thesis under review comprises a collection of comprehensive studies that form a coherent and logically structured whole. The doctoral student's predominant contribution to its implementation, the elevated calibre of the results obtained, and their extensive dissemination in distinguished international journals with a high impact factor (total IF 33.3) merit particular acknowledgement. The doctoral student's intensive scientific activity and grant funding, as well as the fact that he has filed a patent application, which demonstrates the potential application of his research, are also worthy of emphasis.

It is evident that the tasks delineated in the thesis have been comprehensively executed by the author. The findings of the experiments and the conclusions derived from them are both valuable and consistent, and may possess significant practical importance. The author has demonstrated the ability to undertake a research task independently, in line with current scientific trends, conduct advanced experiments, engage in scientific discussion, and formulate substantively correct and well-founded conclusions.

In view of the above, I hereby submit a motion to the Scientific Council of the Food Technology and Nutrition Discipline at the Poznań University of Life Sciences to award a distinction to the doctoral dissertation by Marcellus Arnold, MSc, entitled "Innovative impregnation techniques and process optimisation for shaping the physicochemical, functional, enzymatic, and sensory properties of freeze-dried apple snacks" completed at the Department of Gastronomy and Functional Food Technology under the supervision of Prof. Anna Gramza-Michałowska, PhD.

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