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Review report on doctoral dissertation

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Afiliation: Wrocław University of Environmental and Life Sciences, Faculty of Biotechnology and Food Science

Scientific discipline: Food Technology and Nutrition

Title: Creation of high-quality plant-based powders with targeted health-oriented properties

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Legal basis

The legal basis for this review is the resolution of the Food Technology and Nutrition Scientific Board of Wrocław University of Environmental and Life Sciences dated on September, 26, 2023, assigning me as a reviewer.

The research problem and the scope of the thesis

The current trends in food processing are strongly related to the increasing demand for functional, high

quality food. It is becoming more and more important to offer products rich in native bioactives, and at the same time - with low content of process contaminants. The scope of the dissertation fits well with such trends. It aimed at the evaluation of the effect of the type of matrix and processing conditions for plant-based powders production, on the powders quality. A holistic approach was applied, which included physicochemical and biological characterization. Finally, the recommendations for the final production of powders were defined. Moreover, the dissertation also raises the issue of sustainable management of by-products, in line with the assumptions of the circular economy and "zero waste" trend, because some of the applied raw materials for powders production were fruit pomaces.

Formal evaluation

The dissertation presented for review is written in English. It is a hybrid-type PhD dissertation, consisting of 2 thematically coherent scientific papers, followed by 4 parts of supplementary study, which are all merged into 100-pages (excluding references and appendixes) study. I consider the layout of the doctoral dissertation and the order of chapters appropriate and logic, as it contains the following parts: The list of publications and supplementary studies included in the hybrid doctoral dissertation, Abstract (in English and Polish), Introduction, Hypothesis and research objective, Experimental design, Material and methods, Results, Conclusions, References, Appendices (publications with the declarations[1] of PhD candidate about her role in the preparation of papers, confirmed by the supervisor), Scientific achievements.

The articles were published in Foods, and Food Bioscience. The total impact factor of the publications is 10.127. The attached declarations show the leading role and contribution of PhD candidate in the development of the concept, implementation of the experimental part, the interpretation of the results and the preparation of the publications. I consider the publication of results in high-rated scientific journals as proof of their high level and correctness in terms of research plan, methodology, and

[1] The declaration regarding *Publication 2* was sent separately after the dissertetion was submitted

discussion of results, although I have also some comments and suggestions, connected with my different point of view, as presented below in further parts of review.

The thesis is prepared with very high editing standards. The language is comprehensive and coherent, while errors and inaccuracies are relatively rare.

Evaluation of the content of the thesis

In 3-pages INTRODUCTION PhD candidate presented information about the importance of plant-based powders in the modern food industry, their relation with current trends of circular economy, sustainability, and the quality of life. Also, the most important challenges in the processing of plant raw materials to produce high-quality powders were described, as follows: the appropriate choice of process parameters, undesirable alterations and interactions possible during processing, which can be matrix-dependent and difficult to be controlled. In line with such challenges, this part of the dissertation initially introduces the main aims of the works which were connected with the tracking of processing-induced alterations of native and newly formed components of different botanical matrices, including monitoring the progress of Maillard reactions and caramelization, as well as assessment of powdering technology effect on plant-based powders' biological potential. I find this part of the work highly valuable, as it allows the reader to get acquainted with the most important prerequisites to research and the scope of work.

The next part of the dissertation clearly presents the main HYPOTHESIS, the general RESEARCH OBJECTIVE, and 5 specific objectives, with the indication in which publication/supplementary study the results are included. The whole work was properly planned and arranged. Due to the fact that the scope of work was quite wide and omnidirectional, I appreciate the fact that PhD candidate prepared the diagram to present visually the EXPERIMENTAL DESIGN (Fig. 2). This kind of visual presentation makes it easier to track the investigations. However, what is missing in this diagram (Fig. 2) is the indication of publications/supplementary studies in particular stages, moreover some information are not consistent with the content of the dissertation (they

are listed below in Minor comments section of this review).

The fourth section of the dissertation presents in 7 pages the RESEARCH MATERIALS AND METHODOLOGY applied during investigations. The preparation of feed materials for drying involved many different operations, depending on the raw material and the stage of work: squeezing, extraction, chromatography, formulation with carriers, pasteurization, and fermentation with probiotic strain. What was especially interesting for me, it was the inclusion in the scope of work of some model materials in stage II. Based on the sugar and organic acid profile of Japanese quince and blackcurrant PhD candidate prepared simplified fruit matrix to asses the impact of drying operation on the formation of process contaminants and ascorbic acid degradation. The methods applied for powders preparation and characterization were clearly described. The selection of the methods is correct, some basic, as well as advanced analytical methods were applied to evaluate chemical properties and biological activities. A wide range of statistical methods was applied for data analysis, and a special software was applied for data visualization, which deserves in my opinion a special approval. The methods of visual presentation of are impressive, and give the reader the possibility to track the relationships easier.

The main and the most important part of the dissertation – RESULTS – is presented on 81 pages. It is clearly divided into 3 parts, covering 3 stages of works, each of them divided into 2 parts.

Stage I includes the investigation of processing-induced changes toward improved characteristics of powders obtained from chokeberry and cranberry extracts. Extracts were prepared with different solvents, dried without carriers, and with different types of carriers, by freeze-drying and vacuum drying. Description is based on *Publication 1* and *Supplementary study 1*. Both parts follow similar pattern: first the physical properties of powders are described, followed by chemical properties, divided into phenolic compounds, antioxidant capacity and HMF formation. Finally, chemometric analysis are described and the short summary with practical recommendations are provided. In the case of chokeberry dried powders, the drying technique and its parameters affected the properties to a greater extend than the type of carrier, however

some significant differences between carriers and interplay between two factors were noticeable. The highest content of phenolics along with the lowest HMF content were observed after the application of maltodextrin-trehalose blend, freeze-drying and vacuum drying at 90°C. The same type of carrier was one of the most effective for cranberry pomace extract drying, because it resulted with the powder of high phenolics content. In this study also different types of extraction solvents were tested, and it was proved that acidified 50% ethanol should be considered for the extraction of cranberry pomace, because it resulted with the highest content of phenolics and the lowest HMF formation. Stage I is a valuable source of knowledge about the impact of different types of carriers, extraction solvents and drying methods on the changes in phenolics content and profile, antioxidant capacity and HMF formation in powders. It proves that fruit by-products can be processed to produce high-quality soluble powders that may by potentially incorporated into functional food products. During the analysis of the presented data and description I formulated a few ***questions and comments,*** which should be treated as a voice in the discussion:

1. The total content of phenolics and antioxidant capacity in produced powders should be compared with raw fruits of different botanical origin (based on literature data), or other microencapsulated powders, to show whether the content is at a similar/higher/lower level.
2. Why the raw extracts were not characterized for the content and profile of phenolics, antioxidant capacity and HMF content? Such results could give additional interesting information and possibilities for the discussion of results. Especially, it could help to answer if really during drying the release of some flavonols from more polymerized structures can be observed (as was suggested in *Publication 1*). Moreover, it could help to explain the presence of HMF in freeze dried-powders, which, as suggested (*Publication 1*), might be connected with the particular composition of extract.
3. Describing the content of phenolics, the term "retention" was used many times, which in my opinion should not be used in such a context. If the determinations of phenolics were done only for powders, and there is no comparison between raw material (extracts) and powders, is it only just the content that is presented, not the retention. "Retention" usually is expressed in percentage in comparison to the initial value/level/content.
4. Please try to evaluate what was the actual ratio of extract solids to carrier solids in the obtained powders? It was presented that the extract had 6.9° Brix, and the addition of carrier was 10% (w/w), but it was not specified if this 10% was in the relation to solids of extract. In other words – clarify what was the share of extract solids in powder solids? Based on this - please try to explain why the content of phenolics and the level of antioxidant activity were about 4 times higher in control powdered samples than in powders with carriers (*Publication 1*)? Is it the result of dilution by the carrier? Does it correspond to 4 times higher content of extract solids in powders than carrier solids?

5. Please comment how the addition of carrier affected the formation of HMF (*Publication 1*), compare control samples with the average values for powders with carriers (I am not asking in details how different carriers affected the results, but more generally). In *supplementary study 1* it was concluded that lower level of HMF in carrier-containing powders was connected with lower formation, but maybe it was connected with carrier-dilution effect, similarly as for phenolics content?

Above questions and comments are connected with my different point of view on the set of collected data. In my opinion, inclusion of the comparison between raw material and powders, to define the "real" retention could provide much more additional knowledge and could lead to further interesting conclusions. However, even without such an approach in the data analysis, this part of dissertation in my opinion is of very high scientific standard, and provides a lot of new information, including those which can have practical application.

In **stage II** the aim was to investigate plant-based matrix complexity vs. bioactive response and process contaminants drivers. I found this part of dissertation especially interesting and valuable, because it shows the possibility to design the quality of the powders based on fruit material towards various biological activities. PhD candidate have chosen four materials – Japanese quince, blackcurrant, haskap berry and rosehip, which were dried in the first part of this stage (*supplementary study 2*) by freeze-drying as whole fruits, and after the processing into fractions (juice, pomace, sugar-free juice). I appreciate the explanation showing the scope of this study, in which PhD candidate clarified why this drying method was applied. The main reason was the need to obtain the powders without carriers, to eliminate the possible interactions between components, which can affect biological activity. With such approach, it was possible to trace the particular compounds and biological activities, and their contents and levels in powders produced from different matrices. My main ***comment*** to this part is similar as above – the lack of data for raw materials before drying makes it impossible to describe the changes which can occur during drying. Eventhough freeze-drying was selected, which is a mild low-temperature method, it may cause some alterations and changes, i.e. in amino acids profile (PhD candidate provided such citation - page 58), and in powders it can differ from fresh counterparts (citation on page 56). Thus, it has to be underlined, that the results presented the quality of powders itself, without

knowing the initial state. From practical point of view, it is of course completely correct, because the final user of the powders, while incorporating them into some functional products, will be interested in their activities, not the initial state. On the other hand - this can be interesting from scientific point of view, so I suggest to incorporate also determinations for fresh material in future research, if the topic will be still investigated. I appreciate that PhD candidate did not use term "retention" in this study. The results in this stage were presented in very clear and comprehensive manner, they were divided into sections about particular properties (however, I did not find results of HMF content, while it was listed in the diagram in this stage/study - Fig. 2). In all types of fruits, the highest content of phenolics was in the case of sugar-free juice fraction. The phenolics profile differed between powders of different botanical origin. For example – anthocyanins were not present in Japanese quince and rosehip powders. The part of work describing amino acid profile is particularly interesting and valuable, because it is the first study presenting amino acid profile of powders produced from different fractions of fruits. Among them, the lowest amino acid content was observed for pomace powders, while the powders obtained from sugar-free juices had the highest ratio of essential to non-essential amino acids. It is very interesting knowledge, especially, as was presented in the further part of work, amino acid profile affected the antioxidant capacity. Moreover, some particular amino acids were correlated with antidiabetic activities. Antidiabetic and antiglycation effects were the highest for sugar-free juice powders, and additionally juice and pomace powders from blackcurrant had comparable activity. The second part of stage II (*supplementary study 3*), devoted for drying of model matrices of Japanese quince and blackcurrant juices, focused on the investigation of the formation of process contaminants during drying. It is a very valuable part of the dissertation, because it differs from some previously published studies, which usually focused on sugar and amino acids models susceptible for Maillard reactions and caramelization. Here, the models were composed of sugars and acids, while the target process contaminants were furfural and HMF. Additionally, the degradation of ascorbic acid was studied. HMF and furfural were formed only when vacuum drying was applied, while among carriers - inulin stimulated the formation of contaminants. Moreover, inulin applied during vacuum drying caused the degradation

of ascorbic acid in blackcurrant juice model, while other combinations of carriers and drying methods allowed to retain its concentration. My ***question*** to this part is similar as above – what was the ratio of sugar+acid solids to carrier solids in the obtained powders?

Stage III was related with the biological potential of powdered plant products, as affected by matrix composition and drying process conditions. Blueberry extract of three different cultivars (*supplementary study 4*) and beetroot juice (fresh and fermented) (*Publication 2*) were dried by different methods, with or without carriers (only extracts), and physicochemical and biological properties were evaluated. I asses this part of dissertation as of very high practical importance, because it provides an useful knowledge how the biological activities of powders can be designed, depending on the matrix composition and drying process. Among tested blueberry cultivars, 'Bluecrop' was the richest source of phenolics. The application of inulin resulted in powders of about 2-fold lower phenolics content and antioxidant capacity. Powders did not have antibacterial activity against *E. coli, S. aureus, L. monocytogenes,* while they inhibited the growth of *C. jejuni and H. pylori.* Thanks to chemometric analysis it was concluded that this activity was more related to the presence of some specific phenolics than their total content. This stage of dissertation proved that the selection of plant cultivar, its preprocessing (i.e. by juice fermentation), the selection of carrier material and drying method can be the tools for designing powdered products of programmed biological activities. Such tailor-made approach can be useful for customized food production for different target groups of different needs.

My ***questions*** to this stage are similar as above:

1. What was the ratio of extract/juice solids to carrier solids in the obtained powders? This calculation can be helpful to explain the differences in phenolics content and antioxidant activity between powders with/without carrier. For example, approx. 2-fold decrease of antioxidant capacity and phenolics acids content in powders with carriers was observed (page 89 and 81, respectively), which suggests that the carrier-dilution effect was observed, and that carrier share in powders was about 50% of solids. However, to calculate this share, the value of solids content of extract should be provided, while it is only known that the addition of inulin was 5% (w/w), without the information if it refers to solids. If it refers to the total extract mass, and the extract had about 5% of solids, indeed the approximate ratio of extract solids to carrier solids was 50:50. Similarly - does the ratio of juice:carrier in *Publication 2* (4:1) refer to solids?

2. Lack of data for raw extract/juice hinders drawing the conclusions about the retention of phenolics during drying. In *Publication 2* betalains were determined also in fresh and fermented juice, but these data were not used for the evaluation of retention. In future work, I suggest to calculate the retention coefficient (description published in 2022), which takes into account the initial concentration, concentration in powder and also the share of carrier in powder solids, which taken all together show the level of real retention of selected biocompunds after drying.

I would like to underline, that my comments about the lack of information about the share of carrier in powder solids, and the levels of phenolics content in raw materials, useful for the calculation of retention, do not diminish my positive evaluation of the whole dissertation. They should be treated as a voice in the discussion, and the suggestion for future works. The dissertation is very wide and comprehensive presentation of the possibilities to obtain high-quality plant-based powders, of very high scientific relevance and potential practical applications. What is worth to mention again is the fact that PhD candidate applied a wide chemometric analysis to describe the observed correlations between investigated properties of powders. It helped to form final conclusions and recommendations.

The next part of dissertation, CONCLUSIONS, includes the brief description of the most important outcomes of the work, followed by the statement about the confirmation of hypothesis. This part is clear and logic. However, again I think that the use of term "retention" is not justified, as well as the conclusion about the recommendation to apply the carrier due to lower HMF content, while at the same time the content of phenolics was 4 times lower (compared to carrier-free powders).

Minor comments

The dissertation itself (a study) and the publications being a part of it were prepared with very high editing standard. The structure, layout and visualization of results are clear and comprehensive. Below is a short list of minor editing comments:

1. Title page: the correct name of scientific discipline is Food Technology and Nutrition
2. Figure 2: Stage I and Stage III (part 1) should also have information about drying without carriers; Stage II (part 1) and Stage III (part I) mention HMF content, while it was not

included in the description of results

3. Table 7: typo – the second "No vitamin C" should be replaced with "With vitamin C"?

Final statement

In my opinion the dissertation submitted for review was carefully planned, performed and written. I rate its scientific value as very high. It is an original elaboration of scientific problem, which enriches the knowledge in the field of food technology. Moreover, the results can be useful and used practically. It contains elements of scientific novelty. The PhD candidate demonstrated the ability to conduct in-depth studies, plan and perform experiments, interpret the obtained research results, critically assess facts and formulate conclusions. In my opinion, MSc. Jessica Brzezowska is a scientist fully prepared to conduct independent research.

To sum up, I declare that the PhD thesis submitted for review meets the requirements for doctoral dissertations according to point 187 of the act Law on Higher Education and Science (July 20, 2018, uniform text: Journal of Laws of 2023, item 742, as amended). Therefore, I apply to Scientific Council of the discipline Food Technology and Nutrition of Wrocław University of Environmental and Life Sciences for admission to M.Sc. Jessica Brzezowska to the following stages of the PhD procedure.

Rozprawa doktorska spełnia warunki określone w art. 187 ust. 1-4 ustawy z dn. 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U z 2023, poz. 742 ze zm.).

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Dr hab. Katarzyna Samborska, prof. SGGW