

prof. dr hab. inż. Małgorzata Krzywonos
Wrocław University of Economics and Business
Faculty of Management
Department of Process Management
Komandorska 118/120,
53 –345 Wrocław
email: malgorzata.krzywonos@ue.wroc.pl

Wrocław, 19.08.2023.

Ph. D. Thesis Evaluation Report

Thesis title: **Modification of brewers' spent grain chemical composition to improve their techno-functionality and antioxidant capabilities.**

Author: **Mgr inż Joncer Naibaho**

Supervisors: **dr hab. Małgorzata Korzeniowska, prof. uczelni
Prof. Baoru Yang, PhD**

1. Formal and legal basis for the review

The review was made based on a letter from Chairman prof. dr hab. inż. Agnieszka Kita from the July 14th, 2023, according to the resolution of the Discipline Council for Food and Nutrition Technology of the Wrocław University of Environmental and Life Sciences on June 13th, 2023, regarding the proceedings for the award of a doctoral degree **in** agricultural sciences for the discipline of nutrition and food technology for Mgr inż Joncer Naibaho

The legal basis of this review is the Act of the Law on Higher Education and Science, June 20th, 2018 on academic degrees and academic titles as well as degrees and titles in the field of art (Journal of Laws 2023, No. 742).

2. The selection and meaning of the topic

Food production and processing generate high amounts of waste and by-products. Such by-products have traditionally been rejected and disposed of in landfills due to their quantity and a lack of sustainable management. Industries are now interested in technologies that will lead to zero waste, where the waste generated will be repurposed as the raw material for new value-added products and uses. These actions can directly impact the Sustainable Development Goals, and the Zero Hunger Challenge. The industry might benefit economically from the proper use of food waste and by-products as ingredients or food additives, which would also help to alleviate nutritional issues, promote positive health impacts, and lessen the environmental effects associated with improper waste management.

As part of the circular bioeconomy concept, food processing waste could be used to develop new food ingredients or products for human consumption, promoting their integral valorization and reincorporation into the food supply chain and generating new revenue streams, businesses, and employment opportunities. Plant food processing by-products are a large disposal challenge for the industry. Still, they are also a source of molecules with potential for application due to their advantageous technical or nutritional characteristics.

Suitable levels of proteins, lipids, carbohydrates, micronutrients, bioactive compounds, and dietary fibers make food wastes and by-products of the utmost significance. Knowing how chemicals (or fractions) interact with food is crucial when the extracted particles

are added to meals. The proper solvent selection and extraction procedure are essential, considering the resulting extract's intended use in food. The other operations concerned should also be thoroughly examined.

One of the food processing plant by-products is brewers' spent grain (BSG), which is mainly used as an animal feed for ruminants and fish and as an organic fertilizer. However, its valorization into value-added products is becoming more and more popular.

The subject of the thesis submitted for review (evaluation of the effect of thermal treatment and enzymatic extraction on chemical composition, techno-functionality and antioxidant capabilities of brewers' spent grain) aligns with current research topics in food and nutrition sciences. The research problem undertaken is of great importance, both cognitively and in terms of practical application.

Formal aspects of the work

The doctoral dissertation submitted for review is a thematically coherent collection of six peer-reviewed scientific articles entitled „Modification of brewers' spent grain chemical composition to improve their techno-functionality and antioxidant capabilities”. The collection includes:

Review paper

1. **Naibaho, J.**, Korzeniowska, M. Brewers' spent grain in food systems: Processing and final products quality as a function of fiber modification treatment. *J Food Sci.* 2021; 86: 1532–1551. <https://doi.org/10.1111/1750-3841.15714> (70 p. MES, IF 3,16)

Research papers

2. **Naibaho J.**, Korzeniowska M, Wojdyło A., Figiel A., Yang B., Laaksonen O., Foste M., Vilu R., Viiard E., Fiber modification of brewers' spent grain by autoclave treatment to improve its properties as a functional food ingredient, *LWT*, 2021 149,111877, <https://doi.org/10.1016/j.lwt.2021.111877>. (100 p. MES, IF 4.95)

3. **Naibaho J.**, Aneta Wojdyło, Korzeniowska M., Laaksonen O., Föste M., Kütt M-L, Yang B., Antioxidant activities and polyphenolic identification by UPLC-MS/MS of autoclaved brewers' spent grain, *LWT*, 2022, 163 113612, <https://doi.org/10.1016/j.lwt.2022.113612>. (100 p. MES, IF 6.05)

4. **Naibaho J.**, Bobak Ł, Pudło A., Wojdyło A, Andayani N.S., Pangestika L.M.W., Korzeniowska M., Yang B., Chemical compositions, antioxidant activities and techno-functionality of spent grain treated by autoclave treatment: evaluation of water and temperature levels. *International Journal of Food Science & Technology*, 2023, 58.4: 2130-2140. (70 p. MES, IF 3.61)

5. **Naibaho J.**, Pudło A., Bobak Ł, Wojdyło A, Lopez A. A., Pangestika L.M.W., Andayani N. S., Korzeniowska M., Yang B., Conventional water bath heating on undried brewer's spent grain: Functionality, fatty acids, volatiles, polyphenolic and antioxidant properties. *Food Bioscience*, 2023, 53: 102523. (70 p. MES, IF 5.32)

6. **Naibaho, J.** Korzeniowska M., Wojdyło A., Ayunda H.M., Föste M., Yang B., Techno-functional properties of protein from protease-treated brewers' spent grain (BSG) and investigation of antioxidant activity of extracted proteins and BSG residues. *Journal of Cereal Science*, 2022, 107: 103524 (70 p. MES, IF 5.32)

The papers were published between 2021 and 2023 in journals from the Journal Citation Reports (JCR) list. According to the MES, journals are assigned to the food and

nutrition technology discipline. The total number of MES points calculated according to the papers' publication date equals 550, and the total IF is 27.16.

The original research papers included in the monothematic collection contain a detailed description of the research methodology, conducted experiments, and research results with their discussion and critical commentary against the background of the subject literature, statistical analyses and conclusions presented with clear Tables and Figures.

The works mentioned above are co-authored papers; in all of them, Mr Naibaho is the first Author. His contribution to the original creative work consisted of co-authoring and developing the concept, the original version of the manuscript, investigations, performing the analyses, and analyzing and interpreting the results. Participation in the publications was confirmed by the respective co-authors' statements, except the publication No.2, for which the signed and authors' contribution statements are also not included. In the manuscript of paper 2, authors' contribution credits are present, and the role of Mr Naibaho was conceptualization, Methodology formal analysis and writing the original draft.

All the signed statements of authors' contribution credit were translated into Polish. In some cases, it was not easy to understand the real role of the authors because of the inappropriate wording in this context. For example, the leading role (lead) is translated as "otów" or "trop". Nevertheless, PhD student provided evidence of the leading role in the conception and execution of the research and the preparation of the publications.

The dissertation presented for review, in addition to copies of the published papers (except paper No. 2.) statements of co-authors credits, contains a clear synthesis of the attached publications (33 typed pages). It includes an abstract in Polish and English, an introduction; purpose and the scope of the research; Materials and methods; Results and discussion; Conclusions section; Bibliography (52 references); and Attachments (copy of the publications and authors' contribution statements). It should be mentioned that the thesis does not contain the full text of publication 2, because it was not published in open access.

The bibliography selection is appropriate and includes the most important literature from recent years (only 2 items are from before 2012). The references provided contained full bibliographic data.

Summing up, the dissertation meets the formal requirements for this type of work presented in the procedure for the doctoral degree, i.e., it is experimental and contains all the necessary chapters arranged in a typical sequence. The study is coherent as a whole and is clearly written and presented.

Characteristics and substantive evaluation of the dissertation

The title of the study reflects well the content of the dissertation. The Introduction section starts with the justification of the choice of the dissertation topic, emphasizing the importance and research topic undertaken.

Next, the Author gave a brief description of the chemical composition of BSG in terms of nutritional values that may be useful in designing foods with BSG as an ingredient.

Then, he outlined the state of knowledge and research on the feasibility of using BSG as a food ingredient and the acceptability of BSG-added products. He focused on the mechanism of release of nutritional-related compounds from the BSG matrix after treatment of BSG with temperature and extraction.

When discussing the changes in flavor and sensory effects that water-bath induces (p. 4), for unknown reasons, the Author cited publications on the impact of water-bath on fish,

tilapia and shrimps (Jung et al., 2020; Ye et al. 2022; H Zhang et al. 2015). The biological materials of BSG and fish are different, and the only thing the cited publications have in common with the BSG treatment is the process (water-bath and ultrasound-assisted treatment). To summarise this thread, the Author concludes that the benefits obtained by the cited authors may help improve the BSG properties. *Please explain on what basis the Author came to this conclusion. In my opinion, this is a simplification.*

Later, the Author presented two stages of the study (p.4): first were studies on the influence of autoclave treatment and water-bath heating on chemical composition and techno-functional properties; second on the impact of the different enzymatic protein extraction on polyphenolic compound and antioxidant properties of BSG compared to obtained protein. This is inconsistent with the presentation on the p. 6. The research scheme shows three stages: 1) literature review, 2) thermal treatment and 3) enzymatic-protein extraction. Further, the Authors stated that “a preliminary study was carried out to confirm the hypothesis emphasized in publication 1, applied treatments modify the dietary fiber composition and consequently increase the nutritional compounds”. However, in publication 1, there is no stated hypothesis. Only in the conclusions section is mentioned, “As hypothesised, AT improved the antioxidant properties in BSG including ORAC, ABTS and FRAP compared to that in untreated BSG.” *So Author’s comment is needed.*

In publication 1, the Author stated that: The current study collected and reviewed original research papers that examined and used BSG in food production and were published during the 10-year period from 2010 to 2020. A total of 38 articles were collected from a Scopus and Google Scholar search.” My question is: How were these 38 articles selected? What was the search strategy? What criteria were used? What was the language and type of publications (articles, review technical notes, proceedings)? Why only Scopus and Google and not Web of Science? What were the keywords used for the search? *Please provide the methodology for the searches and the criteria for exclusion and inclusion for the papers.*

In the publication 1 it is: “In this review study, the utilization of distillers’ spent grain (DSG), a cereal byproduct from bioethanol production, is included. BSG and DSG are cereal byproducts that have the same impact in terms of their valorization as food ingredients (Roth, Schuster, et al., 2016).” This paragraph is not precise. *Does this mean that the article included in the dissertation analyzed BSG and DSG? or is it about Roth, Schuster, et al., 2016?*

The authors also stated that “The applications of BSG and DSG in food products include bread, extrudate products, cookies, snacks and crackers, pasta, composite flour, dough, yogurt, and cheese (Abd ELMoneim et al., 2015; Abd El-Moneim et al., 2018; Heredia-Sandoval et al., 2020; Nocente et al., 2019; Sobukola et al., 2013; Torbica et al., 2019).” *Why did the authors analyze DSG when the main goal was to examine the BSG?*

Summing up, the Introduction section is accompanied by well-chosen, up-to-date literature published in English-language journals.

The dissertation aimed to modify the chemical composition, techno-functionality, and antioxidant activities of BSG using various techniques (autoclave heating, water-bath heating and enzymatic treatment). Although the main hypothesis was correctly formulated, no “specific” hypotheses were provided. The statement is that “the research was designed in several phases with different research hypotheses according to their purposes.” A clear aim of each stage is provided. There are two statements about hypotheses in thermal treatment and final study (It was hypothesized, that..); but for preliminary study, it is only articulated as “study aimed to confirm”. I would expect formulation and presentation in a typical

dissertation as main goals, specific goals, and later on, main hypothesis, and specific hypotheses (H1, H2, H3). Now, it is mixed.

In the chapter “Materials and Methods”, the Author presented the biological material used in the study. He claimed in subsection 3.1 that “BSG was stored at freeze temperature for the study”. In paper 2, 3 and 6, it is said that BSG was dried with the convective drying at 75 °C, and after grinding and sieving, it was kept at 4°C. Papers 4 and 5 mention that it was stored at freezing temperature (or -20 °C) before the experiments. *Please clarify how the BSG was stored.*

Next, the research scheme presents a design of the experiments, where stages, conditions, and the main topic of each publication are shown. This section clearly explains the logic that stands for the dissertation. The next section shows the study’s methodology. Still, there is no description of the methods, techniques, or statistics used in the study. In my opinion, *a short description of the methods and techniques used and connected to the publications included in the dissertation will help to understand and follow.* Moreover, the chapter is entitled “Materials and Methods”, and the reader is misled because one receives only the methodology, not the methods description.

The chapter entitled Results and discussion is divided into two subchapters: the first is related to the experiments on the impact of thermal treatment on BSG, and the second is on the effect of the protein extraction on the polyphenolic compounds and antioxidant properties of BSG and obtained protein.

The Author started with an analysis of preliminary studies published in paper No. 2. and paper No. 3. The majority of the treatments increased the amount of soluble dietary fiber (SDF) and decreased insoluble dietary fiber (IDF). Autoclave treatment increases SDF/IDF ratio, which is an important factor in the functional properties of BSG, since DF regulates textures (SDF- soft, IDF- harder). The Author confirmed that AT reduces water-holding and oil-holding capacity of BSG. Studies also confirmed that AT increased swelling capacity. On p. 11 of the dissertation manuscript, it is : “The positive impact if the SDF in regulating textural behavior has also been investigated”. It is unclear who investigated this problem: Author or Foste et al. (2020). *Please clarify.*

Figures should be placed just after the first mention in the text (p. 10-11, Figs 1 and 2), the mention is quite far from the Figures, so it is not easy to analyze it.

In publication No. 2: The authors stated, “AT has been found to slightly increase the mineral content (ash content and Ca) of fish bone with a decrease in protein content (Nawaz et al., 2020).” *Why are Authors comparing fishbone with BSG? Both matrices are totally different. Please comment on this.*

Also, in paper No.2, it is discussed the effect of pH changes on the protein content. Still, the authors do not provide information on how the pH changes after AT treatments during the studies presented in paper 2. *Please comment on this.*

Paper No. 3, it is stated that “(.) the improvement of biological properties of BSG will enhance the quality of BSG both as a food and nutraceutical ingredient. Thus, it will benefit the industry in providing a higher quality of BSG and its derivatives products.” *What is the quality of BSG? How does the Author define the term “BSG quality”? And why is this quality improvement of BSG so important for the industry? Please comment on this.*

During the study, the Author also demonstrated that the phenolic compounds affected dietary fiber degradation due to thermal treatment. The amount of flavan-3-ols decreased by 97% when comparing untreated and autoclaved BSG. Total phenolic acids content increased from 300% to 1000% for 110 and 130 °C, respectively. This difference in

the impact of autoclave treatment on flavan-3-ol and phenolic acids suggests the difference in binding stability between phenolic compounds and polysaccharides during thermal exposure. The increase of the phenolic acids is related to the disruption of cell vacuoles.

In the next subchapter, the Author studied the autoclave heating treatment on fresh undried BSG. Results are published in paper 4. Results show that AT declined the number of ketones, alcohols, and furans and inclined the amount of fatty acids and aldehydes. Several alcohol compounds responsible for fatty odour perception were removed, and some compounds responsible for fruity and hebal perception were formed. The increase in temperature increases the antioxidants' activities (FRAP and ABTS).

In paper No. 4, the authors stated that AH is "simple and easy-to-operate, low-cost instrument". Autoclave treatment needs a relatively lot of energy to heat, and the instrument works under pressure, which might be unsafe for workers. Also, the industrial costs of the autoclave are not so cheap. *Please comment on that.*

Next, the Author analyzed the water-bath treatment on the changes in BSG, and the results of this part are published as paper 5. The amount of flavan-3ols, phenolic acid, and total polyphenolic content on BSG with water-bath heating was lower compared to BSG after AT.

My question to this part is why did the Author chose, as reference papers, Ye et al. 2022 and H Zhang et al., 2015, R Zhang et al., 2018, who investigated surimi and other sea food. *How are surimi, sea food and BSG matrices similar? In which aspects?* Of course, except the same process, which is water bath. Water-bath treatment increased the water-holding and oil-holding capacity of BSG, regardless of time reaction and temperature tested.

In the next stage, the Author presented the results of protein extraction on phenolic compounds and antioxidants' properties of BSG and obtained protein. The obtained results were the basis to publish paper No. 6. Amount of the phenolic acid was discovered to be higher in protein extract than BSG sediments. Total amount of flavan-3ols in BSG protein and sediments were higher than in the untreated BSG. Caffeic acid was not present in both BSG proteins and sediments. Protein fraction possessed higher antioxidant activities (ABTS and FRAP) than BSG sediments.

Finally, the Author has correctly formulated conclusions, presenting his achievements with factual comments.

The articles in the collection have already been published and reviewed, and I also evaluate them very positively. Let me formulate some problems to which I will ask the Doctoral Student to respond/discuss the following:

- It might be used experiment planning methods (DoE), which would have significantly accelerated the work time and reduced the number of experiments performed. At the same time, the Author would have obtained the mathematical form of the objective function with the given input parameters. *I would like to ask the Doctoral Student to propose a plan or plans of the experiment indicating which one might be used, define the objective function, variables and levels to be analyzed. Please also justify your choice.*

- *What limitations did the Candidate recognize during the research, except those already mentioned (more water and energy to re-dry the treated materials when dry BSG was used for experiments)?*

- *What are the further directions of the study regarding the use of BSG and treatment?*

In conclusion, the research presented in M.Sc. Joncer Naibaho's dissertation is an original solution to a scientific problem; it demonstrates that the Candidate has very good general theoretical knowledge of food and nutrition technology. It also proves that he is able

to conduct research independently and prepare articles for publication in international journals.

Summary and final conclusion

The dissertation submitted for review by Joncer Naibaho, M.Sc., presents a high level of content, has significant novel elements and significant application value, and my comments are of a housekeeping and explanatory nature and do not detract from the value of the work. The experimental part was presented clearly and transparently. The research was carried out consistently and using well-chosen, research methods. The statistical analysis of the data obtained was correctly carried out, and the correct interpretation of the results allowed conclusions to be drawn that do not go beyond the results obtained. The Author has improved the formulation of conclusions that correspond with the aim of the research as outlined by the PhD student.

The content of the doctoral dissertation of Mgr inż Joncer Naibaho entitled: "Modification of brewers' spent grain chemical composition to improve their techno-functionality and antioxidant capabilities" and the resulting valuable practical application possibilities give me the right to conclude that it meets all the requirements specified in Act of the Law on Higher Education and Science (Art.187, points 1-4, 20th of June 2018) on academic degrees and academic title as well as degrees and title in the field of art (Journal of Laws No. 742, as amended).

Therefore, with full conviction, I submit an application to the Discipline Council for Food and Nutrition Technology of the Wrocław University of Environmental and Life Sciences for the admission of Mr. Joncer Naibaho to the public defense of this thesis.

