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## **Review Report on PhD Thesis**

**Author: Joncer Naibaho, MSc**

**Author's affiliation: Wroclaw University of Environmental and Life Sciences**

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

**Supervisors: Małgorzata Korzeniowska, PhD, DSc, prof. UPWr  
prof. Baoru Yang**

**Reviewer: Edyta Kordialik-Bogacka, PhD, DSc, prof. PŁ**

### **Context and scope of the thesis**

The objective of the PhD thesis written by Mr. Joncer Naibaho was the assessment of the impact of thermal and enzymatic treatment of brewers' spent grain (BSG) on its chemical composition, functionality, and antioxidant properties.

The topic of the thesis is current and relevant in the context of up-to-date research on BSG potential in the development of functional food and nutraceuticals.

### **General description of the thesis**

The reviewed dissertation by Mr. Naibaho consists of the abstract, in English and in Polish, and seven chapters.

The thesis starts with the *Introduction*, which describes the background to the initiation of the PhD project (4 pages). The second chapter *Purpose and Scope of the Research* (1 page) is the outline of the thesis and highlights research objectives and a scientific strategy. The third chapter *Materials and Methods* (3 pages) is mainly focused on the explanation of the research design, illustrated by a clear and informative figure combining all research stages. This is followed by *Results and Discussion* (15 pages), subdivided in two subchapters: *The impact of thermal treatment on BSG* and *The impact of protein extraction on polyphenolic compounds and antioxidant properties of BSG and obtained protein*. The thesis ends with the *Conclusion* (2 pages), *Bibliography* (8 pages), containing 52 references, and the *Attachment*, which includes the copies of six published peer-reviewed articles.

The series of publications composing a doctoral dissertation consists of thematically related scientific articles:

1. Naibaho, J., Korzeniowska, M. (2021) Brewers' spent grain in food systems: processing and final products quality as a function of fiber modification treatment. *J. Food Sci.* 86, 1532–1551 (review paper)(21 times cited)
2. Naibaho, J., Korzeniowska, M., Wojdyło, A., Figiel, A., Yang, BR., Laaksonen, O., Foste, M., Vilu, R., Viiard, E. (2021) Fiber modification of brewers' spent grain by autoclave treatment to improve its properties as a functional food ingredient. *LWT - Food Sci. Technol.* 149, 111877 (10 times cited)
3. Naibaho, J., Wojdyło, A., Korzeniowska, M., Laaksonen, O., Foste, M., Kütt, M.-L., Yang, B. (2022) Antioxidant activities and polyphenolic identification by UPLC-MS/MS of autoclaved brewers' spent grain. *LWT - Food Sci. Technol.* 163, 113612 (5 times cited)
4. Naibaho, J., Bobak, Ł., Pudło, A., Wojdyło, A., Andayani, S.N., Pangestika, L.M.W., Korzeniowska, M., Yang, B. (2022) Chemical compositions, antioxidant activities and techno-functionality of spent grain treated by autoclave treatment: evaluation of water and temperature levels. *Int. J. Food Sci. Tech.* 16042 (2 citations)

5. Naibaho, J., Pudło, A., Bobak, Ł., Wojdyło, A., López, Á.A., Pangestika, L.M., Andayani, S.N., Korzeniowska, M., Yang, B. (2023) Conventional water bath heating on undried brewer's spent grain: Functionality, fatty acids, volatiles, polyphenolic and antioxidant properties. *Food Biosci.* 53, 102523 (0 citations)
6. Naibaho, J., Korzeniowska, M., Wojdyło, A., Ayunda, H. M., Föste, M., Yang, B. (2022) Techno-functional properties of protein from protease-treated brewers' spent grain (BSG) and investigation of antioxidant activity of extracted proteins and BSG residues. *J. Cereal Sci.* 107, 1–6 (6 times cited)

All papers included in the PhD dissertation were published in highly-impacted refereed scientific journals (*Journal of Food Science* (IF 3.16), *LWT - Food Science and Technology* (IF 4.95 (2021) and 6.05 (2022)), *International Journal of Food Science and Technology* (IF 3.61), *Food Bioscience* (IF 5.32), *Journal of Cereal Science* (IF 4.07)). The total impact factor is very high and equal to 27.16.

All 6 articles were cited (with self-citations) totally 44 times. However, it worth pointing that these papers were published over last three years so the number of citations will probably increase quickly.

Mr. Naibaho is the first author and corresponding author in each of them. The other co-authors provided statements with description of their contributions to the research projects and manuscripts. They showed that Mr. Joncer Naibaho's involvement in the preparation of all papers was significant. Although all original papers were the outcomes of collective work of many authors (from 6 to 9 co-authors) the doctoral candidate significantly contributed to the concept, design, execution, and interpretation of the research studies.

The dissertation contains three figures and one table, which presents the list of the publications included in the thesis.

The structure and format of the PhD thesis are correct and typical. I find the layout of the doctoral dissertation and the order of chapters appropriate and the dissertation itself constitutes a logical and closed whole.

The PhD thesis is well written in clear and concise manner. The language is comprehensive and coherent while errors and inaccuracies are rare.

Mr. Naibaho quoted an appropriate number of bibliography sources.

### Scientific value of the PhD thesis

The topic of the thesis is of high interest from the point of view of current research.

Mr. Naibaho has done a great work analysing large data sets and finding various correlations between specific conditions of thermal and enzymatic treatments of BSG and the chemical composition, techno-functionality, and antioxidant properties of obtained products. He has contributed to a better understanding of the potential utilization of BSG-based products.

The series of publications composing a doctoral dissertation begins with the presentation of the current state of the art related to the nutritional and biological properties of BSG and the valorization of BSG as a raw material to produce food and food supplements (P1). It is worth praising that in the description of the current state of knowledge, Mr. Naibaho cited 125 articles, which fit well the subject of this thesis. The second article (P2) concerns the modification of the BSG dietary fiber composition by autoclave treatment in order to improve its functional properties. The third paper (P3) addresses the impact of autoclave treatment on antioxidant properties and polyphenolic composition of BSG. The next paper (P4) considers the impact of temperature level and water content in BSG during autoclave treatment on the fatty acid composition, volatile compounds as well as water- and oil-holding capacity and antioxidant capacity of autoclaved BSG. In turn, the changes in the fatty acid composition, volatile compounds, water- and oil-holding capacity and antioxidant capacity of BSG resulting from water bath heating are the subject of the fifth article in the series (P5). The last publication concerns enzymatic treatment of BSG and its impact on water- and oil-holding capacity, antioxidant activities, and polyphenolic composition of BSG protein hydrolysates.

I would like to emphasise that apart from the papers included in the thesis Mr. Naibaho is the co-author of other 8 publications, including papers tightly connected to the subject under investigation, for example:

1. Naibaho, J., Korzeniowska, M. (2021) The variability of physico-chemical properties of brewery spent grain from 8 different breweries. *Heliyon* 7, e06583 (14 times cited)

2. Naibaho, J., Pudło, A., Korzeniowska, M., Lu, Y., Yang, B. (2022) Alteration of volatile compounds profile of brewers' spent grain by bath-ultrasonication and its combination with conventional water-bath and autoclave treatment. *Ultrason Sonochem.*, 90, 106192 (14 times cited)

The results presented in the articles included in the series are obtained with up-to-date techniques and appropriate statistical analyses. The results are well presented and the interpretation is at high scientific level. They are reliable and of scientific relevance, which was already confirmed by their publication in international peer-reviewed journals characterized by high, as for this discipline, Impact Factor.

#### Questions and comments:

The reviewer has an easier task since the results of PhD project were published in six papers, and in very good journals, after at least 2 independent referees. However, during the study of dissertation some questions/suggestions appeared:

1. The objectives of the PhD project should be more clearly formulated. The doctoral candidate *aimed to modify the chemical composition, techno-functionality, and antioxidant activities of BSG by several techniques including physical treatments, such as autoclave heating and water-bath heating as well as enzymatic treatments* (page 5). However, he did not specify what he wanted to achieve, what were the desirable characteristics of obtained products.
2. The research hypothesis was not formulated properly since it could be proved without experimentation. According to the author "*the main hypothesis was that different applied methods could alter the chemical compositions and biological properties differently*" (page 5).
3. The doctoral candidate should better emphasize the main points and scientific significance of his research (pages 24-25). That's why I would like to ask the candidate for highlighting the most important findings in the context of existing knowledge and indicating the crucial original achievement during the PhD defense?

4. What did the author mean by stating "*preliminary study aimed to confirm the improvement of phenolic compounds...*" (page 5)
5. The experiment described in the sixth paper (P6) entitled "*Techno-functional properties of protein from protease-treated brewers' spent grain (BSG) and investigation of antioxidant activity of extracted proteins and BSG residues*" consisted in enzymatic treatment of BSG with proteolytic enzymes, separation the liquid fraction and sediment by centrifugation, preparation of extracts with methanol solution and finally the analysis of antioxidant and techno-functional properties as well as polyphenolic identification of the both fractions. I would like to ask the candidate why the liquid fraction was called the protein fraction? Why did he use, in the title and then several times in the text of the paper, the wording "*the properties of protein from protease-treated brewers' spent grains*", "*activity of extracted proteins*", "*the protein generated by protease treatments*", "*the current study revealed that enzymatic treatments enhanced the protein content*"? The proteins from BSG were degraded with proteolytic enzymes and, in addition, the protein hydrolysates were not investigated in terms of the protein (products) analysis? It is worth mentioning that the used enzymatic preparation Flavourzyme<sup>®</sup> is a blend of endo- and exo-peptidases and consists of at least eight enzymes, including aminopeptidases, two dipeptidyl peptidases, three endopeptidases. The other applied enzyme Protamex<sup>®</sup> is very broad-spectrum endo-protease and provides an extensive hydrolysis.
6. The expression "*the protein extracts of BSG (BSGPs)*" relating to the control sample (P6) is also misleading since it is not known what was the composition of these extracts and whether the proteins were present in them.
7. Why was such a small amount of flavourzyme (0.1%) used (P6) if at least 1% of this enzyme was applied for preparation of BSG protein hydrolysates in other studies?
8. Why was the BSG hydrolysis conducted at pH 8.5 (P6) when according to the enzyme producer a working pH range for Flavourzyme<sup>®</sup> is 4-8?

9. How was the protein content determined (P6)? Did the applied method of analysis allow to determine the concentration of proteins or nitrogenous compounds?
10. It is not legitimate to use the expression "*total polyphenolic*" in the table 1 (P6) when it was only a sum of phenolic acids and flavan-3-ols.
11. The BSG used in the PhD project should be characterized thoroughly. It is not sufficient to state that the BSG was collected from a local brewery, light-beer-producer since the chemical composition of BSG is variable depending on raw materials used and the brewing conditions in the brewery.
12. Finally, a minor remark, it is difficult to comprehend what was a real contribution of a co-author based on his/her statement when he/she stated that his/her role was "opieka and danymi (wspieranie)" or "dochodzenie (wspomaganie)".

### **Final Conclusion**

The PhD dissertation of the Mr. Naibaho shows his knowledge of the publications related to the PhD topic and the state of the art in this field. The presented five original articles and the review paper are sufficiently extensive for a PhD thesis. The number of techniques used is impressive and the results obtained are of high quality and at international level. The way of preparing the thesis, including the presentation and interpretation of the results, proves that the doctoral candidate not only has extensive knowledge concerning the subject of research but also analytical skills and ability to draw conclusions from measurement data.

The research results of this PhD thesis contributes significantly to the knowledge of the potential utilization of BSG as raw material in food processing.

Mr. Naibaho demonstrated that he can do research independently and think critically.

Summing up the whole review, it can be concluded that the doctoral dissertation demonstrates the candidate's general theoretical knowledge in a discipline Nutrition and Food Technology and the ability to conduct research independently. The subject matter of the doctoral dissertation is an original solution to a scientific problem. In my opinion, the doctoral dissertation submitted for review meets the requirements set out in Article 187 items 1-4 of the Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2023, item 742).

In view of the above, I hereby apply to the Discipline Council for Nutrition and Food Technology of Wrocław University of Environmental and Life Sciences to admit Mr. Joncer Naibaho, MSc to further stages of the doctoral procedure.

Uważam, że przedstawiona do recenzji rozprawa doktorska spełnia warunki określone w art. 187 ust. 1-4 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2023, poz. 742). W związku z powyższym, przedkładam Wysockiej Radzie Dyscypliny Technologia Żywności i Żywnienia Uniwersytetu Przyrodniczego we Wrocławiu wniosek o dopuszczenie mgr Joncera Naibaho do dalszych etapów przewodu doktorskiego.

Edyta Kondziel - Bogacka