Lublin, 24.04.2025

Michał Świeca, PhD, DSc, Prof.
Department of Biochemistry and Food Chemistry
University of Life Sciences in Lublin
michal.swieca@up.lublin.pl

Review Report on Doctoral Thesis, PhD candidate: Zhe Chen.
Thesis title: Application of supercritical carbon dioxide to improve the quality of ready-to-use carrots and pumpkins during storage.
Supervisor: Prof. dr hab. inż. Krystian Marszałek, Prof. Waclaw Dabrowski Institute of Agricultural and Food Biotechnology – State Research Institute, Department of Fruit and Vegetable Product Technology.
Second Supervisor: Prof. Dr. Zhenzhou Zhu, Wuhan Polytechnic University, School of Modern Industry for Selenium Science and Engineering

Legal basis: Act of 20 July 2018 - Law on Higher Education and Science (Journal of Laws of 2023, item 742, as amended)
This review was carried out in response to Prof. Dr Hab. inż. Stanisław Ptasznik (Vice Chair of the Scientific Council of IBPRS-PIB) invitation from the 18th of April 2025.

Justification for undertaking the research topic and formal evaluation of the work

The market for ready-to-use and low-processed food has been developing extremely dynamically in recent years. Due to the specific technological processing chain and storage requirements, maintaining the quality of these products is extremely difficult. At the same time, increasing consumer awareness also limits the use of conventional chemical preservatives and aggressive physical treatments. This situation forces food producers to develop new or adopt

already existing approaches, which not only ensure food safety but also help maintain high nutritional value and potentially enhance health-promoting properties. These solutions should, at the same time, cover three main aspects: (1) limiting enzymatic browning, (2) reducing microbiota growth, and (3) ensuring unchanged or improved nutritional quality. In this context, the application of supercritical carbon dioxide (SCCD) to prolong the shelf life of ready-to-use vegetable-based products appears to be a promising solution. As an eco-friendly and cost-effective technique, SCCD treatments can be successfully integrated into modern food processing technologies.

The dissertation has been prepared following the principles established for doctoral theses (paragraph 3, Article 187 of the Law). The work begins with abstracts in both English and Polish (in line with paragraph 4, Article 187 of the Law), which briefly present the research background, main results, and conclusions. Next, the Author presents a table of contents and a list of publications included in the doctoral dissertation. Accordingly, the basis of the doctoral dissertation by MSc Zhe Chen, titled "Application of supercritical carbon dioxide to improve the quality of ready-to-use carrots and pumpkins during storage", consists of the collection of four papers published between 2022 and 2025 in renowned journals traditionally publishing the studies covering aspects related to food technology for human nutrition:

[P1] Chen Z., Spilimbergo S., Khaneghah M. A., Zhu Z.Z., Marszałek K. (2022). The effect of supercritical carbon dioxide on the physiochemistry, endogenous enzymes, and nutritional composition of fruit and vegetables and its prospects for industrial application: An overview. Critical Reviews in Food Science and Nutrition, 64(17), 5685-5699.

[P2] Chen Z., Kapusta I., Zhu Z. Z., Marszałek K. (2024). Enzyme activity and nutritional profile of different-sized carrot cubes treated with supercritical carbon dioxide. Postharvest Biology and Technology, 210, 112763.

[P3] Chen Z., Kapusta I., Zhu Z. Z., Marszałek K. (2024). Quality properties and nutritional compounds of fresh-cut pumpkin treated with supercritical carbon dioxide. The Journal of Supercritical Fluids, 206, 106147.

[P4] Chen Z., Zhu Z. Z., Marszałek K. (2025). Changes in the storage quality of fresh-cut vegetables using supercritical carbon dioxide treatment. Food Chemistry, 465, 142131.

All publications are multi-author papers in which Mr. Chen is listed as the first author and his contribution, as well as other co-authors, has been clearly described and confirmed in the

attached declarations (pages 119-152). According to the provided information, the PhD candidate was involved in developing the research concept, planning the experiments, selecting research methods and conducting the experiments, analyzing and interpreting the results, as well as preparing both the initial and final versions of the manuscripts.

It is also worth noting that the studies were supported by the project "Innovative high-pressure process to increase the preservation of "ready to eat" organic food" granted in ERA-NET SUSFOOD2 and CORE Organic Cofunds Joint Prof. Waclaw Dabrowski Institute of Agricultural and Food Biotechnology – State Research Institute Call 2019: "Towards sustainable and organic food systems" granted by the National Center for Research and Development.

Next, Mr. Chen presents a critical literature review highlighting the key aspects related to the application of SCCD in food systems (chapter Literature Review, pages 17-26), aims and hypothesis (chapter Hypotheses, Purpose, and Contents, page 27), the materials an methods (chapter Materials and Methods, pages 29-34) as well as the most important achievements and their critical discussion (chapter Results and Discussion, pages 35-46), and a final summary (chapter Observations and Conclusions, pages 47-48). The bibliography list contains as many as 176 literature items published mainly in recent years in journals that traditionally publish research in food technology and nutrition. One of the last chapters (pages 153-165) evaluates the PhD candidate`s scientific achievements to date, which include, among others, 20 scientific publications (total IF 115), numerous conference reports, a list of realized projects, awards and distinctions.

Academic evaluation of the dissertation

The academic evaluation covers both the dissertation and the manuscripts that are part of it. Since the research findings have already undergone peer review during the publication process, this section focuses primarily on their significance for the advancement of knowledge, with only limited reference to specific numerical data.

The aspect of SCCD application was discussed in detail in the chapter Literature Review, publication P1 and the Introduction section of other manuscripts. The review discusses the advantages and limitations of this technology, with particular emphasis placed on consumers' quality parameters (colour preservation), food safety (reduced microbiological spoilage) and nutritional quality (antioxidant stability, enhancement of health-promoting properties,

maintaining nutritional value). This section provides a comprehensive summary of the current state of knowledge and situates the doctoral project within this scientific context.

On page 20 (chapter 1.2.2.) Mr Chen shows a potential mechanism of antimicrobial action of SCCD, listed, inter alia, a decrease of pH causing "destabilisation of the quaternary structure of endogenous enzymes by disrupting hydrophobic interactions and breaking hydrogen bonds". On the other hand, this mechanism is omitted in terms of enzyme inhibition (especially oxidase with optimum pH at higher values). I would appreciate a comment on this matter. Additionally, in chapter 2.4. (page 23) a different behaviour of phenolics in the vegetable/fruit samples treated with SCCD has been presented (unchanged, 2-fold increase, and even significant reduction). Could you suggest any explanation for this phenomenon?

The dissertation presents clearly defined research hypotheses, accompanied by a description of the research scope designed to verify them (also supported by graphical illustration). However, in this place, I would like to ask how you understand the statement "…increase the measured concentration of bioactive compounds…Do you mean the content? (hypothesis 2).

The materials used, the SCCD treatment procedure, and the research methodology are generally described well. Although in some sections important details are missing (e.g. homogenization method in the section 3.3., missing refences in the section 3.6) or the protocols are presented in a rather concise manner (of course full version allowing the repetition of the experiments are in the manuscripts but if the decision was made to add full descriptions it should be made with appropriate thoroughness). Referring to this part, I would like to ask about the choice of extraction conditions selected for the oxidases study (1:1 ratio) or phenolic antioxidants (1:2 ratio). In the PhD candidate's opinion, did they ensure effective extraction?

The dissertation also contains a chapter "Results and Discussion" (pages 35-46), in which the Author presents the main findings of the doctoral project, referring them to the manuscripts constituting the basis of the dissertation. The results confirm the usefulness of the SCCD treatment in maintaining (in some cases also improving) some features of carrot and pumpkin cubes during storage. What is important, this eco-friendly technique effectively reduces the growth of endogenous microbiota and at the same time has a positive impact on the antioxidants and colour of the studied ready-to-use product. Mr. Chen demonstrated (P2, P3) that the successful application of SCCD in the food industry requires prior optimization. Using statistical methods

(principal component and correlation analyses), a theoretical basis for this treatment was established. Key variables such as sample size, temperature, processing time, and SCCD pressure were considered. As presented, the observed effects varied significantly depending on the processing conditions, and in many cases, the final product showed a deterioration in certain quality attributes. In this context, the identification of optimal conditions for the treatment of carrot and pumpkin cubes is particularly valuable. The selected conditions not only preserve the quality of the raw materials but also enhance certain desirable properties, such as increasing antioxidant potential. As a reviewer, I would like to point out the incorrect classification of ascorbic acid as a phenolic compound (Table 2, P2) and ask about relatively low contents of individual phenolic acids determined in the materials.

A logical continuation of the optimization studies was the analysis of the impact of the applied technology on product quality during storage. The results presented in P4, evaluated the effect of the SCCD treatment on the oxidases activity, color characteristic, microbiological quality, low-molecular weight antioxidants content and resulted antioxidant activity of fresh-cut pumpkins and carrots subjected to cold storage. The results demonstrated that SCCD effectively reduce total microbial growth (there are some inconsistent in terms of CFU expression – CFU vs lg CFU), and maintain the low activities of polyphenol oxidase (PPO) and peroxidase (POD) during storage. Although the direct effects generated by oxidases (enzymatic browning) do not appear to be significant in originally orange-colored matrices, a reduction in these enzymatic activities is still reflected in the kinetics of phenolic compounds (oxidase substrates) and changes in antioxidant potential during storage. In this section, a potential mechanism for the increased carotenoid content is also proposed. However, in my opinion, without studies investigating changes in metabolic pathways (enzymes activity, genes expression) or the levels of intermediate compounds, such a hypothesis remains speculative. At this stage, I would rather attribute the observed increase to enhanced extractability, likely resulting from structural changes in the product induced by SCCD treatment. Finally, the doctoral project is summarised in the chapter “Observations and Conclusions” (it was prepared in the descriptive form), where Mr. Chen the findings to previously proposed research hypotheses. Unfortunately, this section primarily focuses on presenting the results, while lacking the broader conclusions and recommendations that would typically be expected in this place. Therefore, I would like to ask: how do you position your findings within the context of existing knowledge, and which of them do you consider the

most significant or valuable? Out of scientific curiosity, I would also like to ask whether the use of SCCD induced any structural changes in the products. Were such studies conducted as part of the doctoral project? If not, could the PhD candidate elaborate on this aspect?

Of course, every doctoral project solves a specific scientific problem (fully addressed in this dissertation; paragraph 2, Article 187 of the Law), but it is equally important that it inspires the formulation of new questions, thereby paving the way for future research directions. The dissertation submitted for evaluation undoubtedly falls into this category. The presented research on the application of SCCD for improving ready-to-use vegetables opens new possibilities in the design of food preservation processes. Based on the analysis of the theoretical parts of the doctoral dissertation and manuscripts, the key role of PhD candidate in project implementation as well as his current scientific activity, I can unequivocally state that he possesses the skills necessary to conduct independent scientific research and has a solid theoretical foundation in the discipline of food and nutrition sciences (paragraph 1, Article 187 of the Law). Mr. Chen has skillfully integrated topics from food technology, analytical chemistry, and related sciences, and the results obtained are valuable both in terms of cognitive insights and potential applications. At this point, I would like to emphasize that the few comments and suggestions provided in my review are meant for discussion and do not alter the high substantive evaluation of the research.

Final Conclusion

The reviewed doctoral dissertation by Mr. Zhe Chen "Application of supercritical carbon dioxide to improve the quality of ready-to-use carrots and pumpkins during storage", meets the requirements set out in Article 13, paragraphs 1–4 of the Act of July 20, 2018 – Law on Higher Education and Science (Journal of Laws 2023, item 742, with later amendments). Therefore, I recommend to the Scientific Council of the Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology that its Author be admitted to the subsequent stages of the doctoral proceedings.

Signed by / Podpisano przez:

Michał Świeca
Uniwersytet Przyrodniczy w Lublinie

Date / Data: 2025-04-24 08:36

Michał Świeca, PhD, DSc, Prof.