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# REVIEW

## Doctoral thesis of Mr. MSc Zhe Chen entitled: „Application of supercritical carbon dioxide to improve the quality of ready-to-use carrots and pumpkins during storage" carried out at Prof. Waclaw Dabrowski Institute of Agricultural and Food Biotechnology – State Research Institute under the supervision: Mr. Prof. Dr. Hab. Eng. Krystian Marszałek (Supervisior), and Mr. Prof. Dr. Zhenzhou Zhu (Second Supervisor)

*Formal basis: letter from Prof. Dr. Hab. Eng. Stanisław Ptasznik (Deputy Chairman of the Scientific Council of IBPRS-PIB) dated March 18, 2025, informing about resolution No. X/184/2025 of the Scientific Council of Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology—State Research Institute in Warsaw regarding the appointment of reviewers for the doctoral thesis of Mr. MSc Zhe Chen*

### Introduction—justification for undertaking the research topic

"Ready-to-use" food is a response to the needs of the modern consumer, who, in addition to attractive sensory and physicochemical properties of products, also expects convenience in their consumption, but most importantly, preservation of their original chemical composition and health-promoting properties. Thus, the modern food industry, in addition to the generally understood food safety, must also focus on meeting consumer requirements in terms of the high quality of the food they produce. Traditional preservation methods, often based on high-temperature processing such as pasteurization, sterilization, or drying, although effective in ensuring microbiological safety, often lead to significant losses in nutritional value, deterioration of sensory properties, and degradation of bioactive compounds.

In response to these problems, there is growing interest in modern, nonthermal processing technologies that allow the preservation of the natural properties of fruits and vegetables while limiting the development of microorganisms and enzymatic activity. One of the promising methods is processing using supercritical carbon dioxide (SCCD). This technology allows food to be processed at low temperatures and without the use of chemicals, which makes it environmentally friendly and attractive from the point of view of the food industry. It combines the effectiveness of preservation with a minimally invasive effect on the chemical composition of products developed based on plant raw materials. However, it is little known in the context of its application in the preservation of low-processed food, including ready-to-use forms. Of particular interest in this context is the effect of using SCCD on the quality of plant tissue, physical and sensory properties, as well as the chemical composition and health-promoting properties of fresh raw materials, because, so far, the application of SCCD has not been considered on such a wide scale.

**Therefore, it should be stated that the research problem indicated by the PhD student and its relevance from the point of view of current knowledge and its deficits fully justify taking up the topic in the assessed dissertation. The issues discussed in the work constitute an original study consistent with growing consumer awareness and the need to introduce changes in production processes. They also provide an opportunity to increase the profile of SCCD use as an alternative method for high-temperature food preservation, which, in the context of food production, seems extremely promising.**

**Assessment of the work arrangement and compliance with formal requirements**

The doctoral dissertation by MSc Zhe Chen, entitled "Application of supercritical carbon dioxide to improve the quality of ready-to-use carrots and pumpkins during storage" is a sequence of four thematically coherent works—one review and three original research papers—published between 2022 and 2025 in renowned international journals. The dissertation includes the following publications, with a total impact factor of 29.5 and, according to the list of scoring journals of the Ministry of Science and Higher Education, 640 points:

1. 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. doi:10.1080/10408398.2022.2157370.
2. 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. doi:10.1016/j.postharvbio.2024.112763.
3. 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. doi: 10.1016/j.supflu.2023.106147.

4. 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. doi:10.1016/j.foodchem.2024.142131.

The assessed work comprises a 165-page study containing copies of four co-authored publications, in which the PhD student played a leading role (as first author), as confirmed by attached declarations of the contributions of the other authors. The structure of the assessed work is correct and consistent with the formal requirements for doctoral dissertations. The series of scientific publications submitted for review is preceded by an introduction, followed by the research hypotheses, aim of the work, and scope of the research. The material and research methodology are also clearly presented. The PhD student then discusses the most important results, referring to specific publications from the series. Finally, the work includes a summary and a bibliography consisting of 161 carefully and accurately selected current literature items. The dissertation also includes summaries in English and Polish and information about other scientific achievements. **Therefore, to sum up the formal assessment of the dissertation, I conclude that it meets the requirements for the degree of doctor.**

**Aim of the work and its justification**

The aim of the work is preceded by a theoretical introduction, in which the PhD student presents the current state of knowledge related to the topic of the doctoral thesis, and identifies areas requiring further exploration and deeper analysis. In the introduction, the PhD student refers to the first publication in the presented series—a review article (*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*)—and discusses in detail the potential of SCCD application in the food industry, particularly its effects on the physicochemical characteristics and chemical composition of fruit and vegetable products. The introduction, together with the review article, is carefully constructed and forms a logical whole, serving as a reference to the aim and scope of the work. It is a pity, however, that the PhD student did not include an economic analysis of the SCCD process at this stage. Moreover, although the review article points to certain problems, such as maintaining the texture of plant materials after SCCD treatment, the student does not further analyze other threats associated with the use of this method.

Despite this, it is evident from the outset that the PhD student is well-versed in the subject and moves freely in the subject matter. In addition, it should be objectively noted that the introduction and the conclusions of the first article directly relate to the specific objectives, which the PhD student formulated as:

1. Assessment of changes in enzyme activity and the nutritional profile of different-sized carrot cubes treated with SCCD.

2. Evaluation of the influences of SCCD processing on the physicochemical properties, phenolic compounds, carotenoids and sugar profile, and antioxidant activities of pumpkin cubes.
3. Measurement of changes in the carotenoid and sugar profile, phenolic compounds, and antioxidant capacity of carrots and pumpkins during storage.
4. Revealing the quality maintenance mechanism of the SCCD technique on bioactive compounds in carrots and pumpkins during storage.

The PhD student also proposed four research hypotheses. It should be noted that, in the subsequent stages of the dissertation, the Master consistently implemented the defined objectives and tested the proposed hypotheses. However, it is regrettable that the student did not define one overarching research objective, which could then be elaborated through the specific goals. In addition, the graphical representation of the research content could be enriched by incorporating the objectives and hypotheses to enhance clarity. The graphic is also missing a caption.

Nevertheless, this does not affect the overall assessment that **both the objectives and the research hypotheses were appropriately formulated and aligned with the content of the dissertation and the publications included in the series.**

**Evaluation of the experimental part of the work**

The "Materials and methods" chapter—both in the description and in the individual publications (from 2 to 4)—allows for an assessment of the methodological approach to the experiments planned in the doctoral thesis. The first part focuses on presenting the materials and describing the conducted experiments, namely SCCD processing for carrots and pumpkins, and the subsequent storage experiment. In the second part, MSc Zhe Chen provides a fairly detailed description of the analytical and statistical methods used. **The research methodology is well presented, and upon review, it can be concluded that the experimental approach—particularly the selection of analytical methods for the physicochemical evaluation of the processed formulations—was appropriately structured.**

However, three fundamental questions arise:

1. Why did the PhD student choose to test specifically these sizes—1 cm cubes (carrots and pumpkins) and 2 cm cubes (carrots)? Is there a practical justification for this choice? Why were two size variants not tested for pumpkin tissue? While SCCD may have proven more effective with 1 cm carrot cubes, pumpkin represents a different type of plant tissue. Using two sizes would have either confirmed or challenged previous observations.

2. In the case of enzyme activity analysis (PPO and POD), would it not have been more appropriate to crush frozen samples (−80°C) using an impact mill? Grinding with a blender for 5 min could initiate enzymatic oxidation, potentially falsifying the results.
3. Why was it decided to determine polyphenolic compounds using the nonspecific Folin–Ciocalteu method? Simultaneously with the qualitative analysis of polyphenolic compounds using chromatographic techniques (UPLC-PDA-MS/MS), a more reliable quantitative analysis could be performed.

In addition, there is a suggestion that in future studies, a less volatile organic solvent could be used in the final stage of carotenoid content analysis instead of acetone. Acetone evaporates very quickly, which may lead to sample concentration due to evaporation, even under refrigerated conditions in the autosampler.

**Nonetheless, these questions do not affect the overall assessment that the quality of the experimental part of the work was at a very good level.**

**Interpretation and discussion of the obtained results and evaluation of the conclusions**

**After reading the dissertation of MSc Zhe Chen and the results of the research he conducted, it should be stated that the work is interesting and addresses novel issues in the field of food technology and nutrition. The title of the doctoral dissertation accurately reflects its content, and the fact that the research results have been published in indexed scientific journals with global reach ensures that they have undergone detailed substantive evaluation.**

The PhD student published a total of three research manuscripts, each addressing the topic of SCCD use in the preservation of carrots and pumpkins, as well as the analysis of the effects of SCCD on the storage quality of these vegetables. In the article entitled "*Enzyme activity and nutritional profile of different-sized carrot cubes treated with supercritical carbon dioxide*", he analyzed the effect of SCCD treatment on carrot cubes cut into 1 and 2 cm sizes. The samples were subjected to SCCD under different combinations of pressure (10 and 30 MPa), temperature (35 and 55°C), and time (15 and 45 min), and the resulting formulations were evaluated for physicochemical properties, including enzyme activity (PPO and POD), bioactive compound content, and antioxidant properties.

In the article entitled "*Quality properties and nutritional compounds of fresh-cut pumpkin treated with supercritical carbon dioxide*," the PhD student assessed the effects of SCCD on freshly cut pumpkin cubes, focusing on enzymatic activity, color, bioactive compound content, and antioxidant activity. As in the carrot experiments, the same combinations of pressure, temperature, and time were used, but only one cube size—1 cm—was tested. The choice of a single size is likely justified by the results obtained in the first stage of SCCD testing on carrots. However, considering that pumpkin is a different plant tissue, it would have been valuable to test two size variants (1 and 2 cm) for pumpkin as well, to either confirm or challenge earlier observations.

In the final article of the series, "*Changes in the storage quality of fresh-cut vegetables using supercritical carbon dioxide treatment*" the PhD student examined the physicochemical properties and microbiological stability of SCCD-treated carrots and pumpkin during a 21-day storage period. For this purpose, vegetables were treated with SCCD at 45°C and 10 MPa for 20 min, and then stored at 4°C for 3, 7, 11, 15, and 21 days. At this stage, a question arises: Why were these particular SCCD parameters chosen for the investigation of storage changes in carrots and pumpkins?

The interpretation of the results obtained from all the conducted experiments allowed the PhD student to observe certain relationships and trends in seven key areas:

(i) **Color.** In the case of carrots, SCCD treatment improved lightness ($L^*$) and reduced browning, especially at lower temperatures (35°C). However, higher temperatures (55°C) caused pigment degradation and a decrease in the $a^*$ and $b^*$ values. In contrast, for pumpkin, a decrease in lightness ($L^*$) was observed, along with an increase in color saturation ($a^*$ and $b^*$), indicating better carotenoid release at higher pressures and temperatures. The PhD student concludes that SCCD can positively affect the appearance of fresh vegetables, particularly pumpkin.

(ii) **Microbial activity.** Following SCCD application, the number of microorganisms in carrots and pumpkins dropped below 1 log unit and remained at a low level even after 2–3 weeks. Yeast and mold levels in SCCD-treated samples remained below detectable limits throughout the storage period. The PhD student concludes that SCCD effectively reduces microbial activity and extends the shelf life of fresh-cut vegetables, thanks to the ability of $CO_2$ to penetrate microbial cells and disrupt their cellular metabolism.

(iii) **Enzyme activity.** SCCD processing of carrots and pumpkins resulted in a gradual decrease in the activity of PPO and POD enzymes, with increasing temperature, pressure, and processing time. During the 3-week storage period, enzyme activity in treated samples increased more slowly and reached lower peak values than in untreated controls. The PhD student concludes that SCCD not only partially deactivates enzymes but also limits oxygen access in the tissue, thereby effectively slowing enzymatic browning and qualitative degradation.

(iv) **Sugar content.** As a result of SCCD treatment, the sugar content in carrots varied depending on cube size and processing parameters. In smaller cubes (1 cm), sugar content decreased, while in larger cubes (2 cm), it initially increased and then declined with rising temperature and pressure. In pumpkin, increased temperature and pressure during SCCD led to sucrose hydrolysis, resulting in a decrease in sucrose content and a corresponding increase in glucose. This transformation was facilitated by the acidification of the environment due to $CO_2$ dissolving in water. The PhD student also notes another observation: sorbitol, initially absent in the samples, appeared after 3 days of storage—formed from glucose through catalytic hydrogenation. In SCCD-treated samples, sorbitol levels consistently increased and remained significantly higher than in untreated samples, indicating SCCD's role in stimulating the metabolism of its precursors.

(v) **Polyphenolic compounds**. SCCD treatment of carrots and pumpkins led to an increase in polyphenolic compound content compared to control samples, including qualitative improvements. However, prolonged exposure to high temperatures caused degradation of these compounds. As indicated by the PhD student, the high concentration of polyphenolic compounds in the optimal conditions of the SCCD process was the result of increased permeability of cell walls, as well as the disruption of hydrogen bonds between polyphenolic compounds and other compounds due to the penetration of $CO_2$ into the raw material tissue. The MSc student also indicated that the polyphenolic compounds contained in the raw materials discussed were significantly more stable during the storage process than the control samples, which indicates the high potential of SCCD in the processing of raw materials with a high concentration of bioactive compounds.

(vi) **Carotenoids content.** SCCD technology can effectively enhance the extraction and stability of carotenoids in plant materials, provided that process conditions are appropriately selected. Excessively intense parameters may lead to degradation of these valuable compounds. The PhD student also observed that SCCD-treated samples retained higher carotenoid levels during storage compared to controls, suggesting that SCCD inhibits carotenoid degradation. This effect likely results from limited oxygen access, reduced oxidizing enzyme activity, and possible of stimulationcarotenoid biosynthesis.

(vii) **Antioxidant acitivity.** The PhD student identified a positive correlation between polyphenolic compound content and antioxidant activity, again underscoring the importance of selecting optimal SCCD processing parameters.

**To sum up the core of the presented dissertation, it should be emphasized that the PhD student demonstrated significant commitment, considerable effort, and strong competence in the analytical techniques employed. The results obtained by Mr. Zhe Chen were thoroughly discussed and compared with the latest literature in the subject. It should also be noted that the author consistently and successfully achieved the stated objectives of the work and verified the research hypotheses. The culmination of the dissertation is the chapter "Observations and conclusions," which is precise and well-supported by the results. These conclusions also have practical application value, which adds to the significance of the findings.**

## Conclusion

The doctoral thesis submitted for evaluation presents an original and valuable scientific study that significantly expands both national and international knowledge regarding the use of SCCD as a minimally invasive and low-temperature method for processing plant raw materials. This approach ensures the preservation of high-quality physicochemical properties of the materials. The issues addressed in the dissertation represent an original contribution aligned with rising consumer awareness and the growing need for innovation in production processes. The research results obtained are highly promising and possess practical application value, which adds to the significance of the study. The work conducted by MSc Zhe Chen, along with its quality, demonstrates the PhD student's strong substantive and analytical competence. The results have high cognitive value and make an original contribution to the advancement of knowledge in the fields of food technology and nutrition.

**In light of the above, I affirm that the doctoral dissertation by MSc Zhe Chen, submitted for review and entitled "Application of supercritical carbon dioxide to improve the quality of ready-to-use carrots and pumpkins during storage" meets all formal requirements outlined in the Act of July 20, 2018—The Law on Higher Education and Science (Journal of Laws of 2023, item 742, as amended).**

**For this reason, I submit a motion to the Scientific Council of the Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology—State Research Institute in Warsaw to accept the dissertation and admit its author to the next stages of the doctoral process.**

**At the same time, the high scientific standard of the published research motivates me to submit to the High Scientific Council a request for the dissertation to be considered for distinction, by the practices of the Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology—State Research Institute in Warsaw.**

**Paulina Nowicka, PhD, DSc Eng. Associate Professor**

Signed by /
Podpisano przez:

Paulina Balbina
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