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WYDZIAŁ BIOTECHNOLOGII I NAUK O ŻYWNOŚCI
KATEDRA ROZWOJU FUNKCJONALNYCH PRODUKTÓW ŻYWNOŚCIOWYCH

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Ph. D. Thesis Evaluation Report

Thesis title: **Identification and physicochemical evaluation of lysozyme after its thermo-oxidative modification with microwave radiation**

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The review was made on the basis of the resolution of the Scientific Council of the Food and Nutrition Technology Discipline of the University of Life Sciences in Poznan on October 21, 2021 in connection with the procedure of the award of the degree of doctor of philosophy in agricultural science for MSc. Tianyu Yang.

The selection and meaning of the topic

Lysozyme (E.C. 3.2.1.17), one of the most important biologically active egg white protein (the most popular and abundant resource), found also in other animal and plant sources, is known as powerful polysaccharide-hydrolyzing enzyme with the bacteriolytic and mucolytic activities. By hydrolyzing peptidoglycans present in bacterial cell walls, consisting of alternating β-1-4 linked residues of N-acetylglucosamine and N-acetylmuramic acid, lysozyme is able to inactivate and kill Gram positive bacteria. However it has been extensively studied since its discovery by Fleming in 1922, there is still space for novel and scientifically soundness work, mostly focus on the structural modification of the lysozyme molecule to achieve a wider spectrum of a lytic activity especially toward Gram negative bacteria, which are among the most significant public health problems in the world due to the high resistance to antibiotics. Moreover, selected modifications of the lysozyme structure, by chemical and physical factors, including thermal and microwave treatment, can result also in enhanced functionality of the

protein and expand its applications in various food products. That is why optimization of the microwave treatment of hen egg white lysozyme can complement the theoretical knowledge on this protein, as well as widen its practical application.

Formal aspects of the work

The Ph.D. thesis presented by MSc. Tianyu Yang entitled: "Identification and physicochemical evaluation of lysozyme after its thermo-oxidative modification with microwave radiation" consists of 83 pages divided to the following parts: Abstract, Introduction, Purpose and scope of research, Results and discussion, Unexpected effects in microwave-modified lysozyme after its 12-month refrigerated storage, Conclusions, Bibliography, Attachment divided to the List of publications constituting the doctoral dissertation, Publications of the doctoral dissertation, Statements of the co-authors of the publications of the doctoral dissertation. Introductory part relates to the first publication, that is the complete review paper on lysozyme published in very highly ranked (IF=12,563, 200 MEiN points) journal Trends in Food Science and Technology. After one-page chapter stating the research hypotheses and aim, four parts with short description and main findings of the experimental works are presented. All these parts are reflecting the four separate, published in recognized scientific journals, papers in which MSc. Yang is first author (except paper no 5 in which Mr. Yang is a second author). Conclusions together with general remark and the list of cited publications are closing the substantive part of the thesis. Additionally, two statements signed by co-authors of selected publications are attached.

The assessed 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 a coherent whole and is correctly written in clear and understandable language.

Content of the work

Introduction – Prepared on the base of **the first publication** included in the doctoral dissertation (Lysozyme and its modified forms: a critical appraisal of selected properties and potential, *Trends in Food Science and Technology*, 2021, 107, 333-342) introductory section is concise but informative. It brings crucial information about lysozyme, its natural sources and selected biological properties. In two paragraphs Author pointed out the possibilities of chemical, thermal and membrane techniques modifications of lysozyme solutions and positive effects on selected activities and properties of the enzyme. In addition some other methods of

structural modification of proteins are mentioned with an clear indication of possible positive influence of microwave radiation on lysozyme properties.

Purpose and scope of research – Based on the literature data MSc. Yang has formulated four research hypotheses as a starting point for drawing the aim of the study. In short, he claimed that microwave radiation, assisted by oxidation, can positively modify lysozyme functionality, which can be further changed by storage conditions. However, hypotheses are informative and show the most important assumptions for the study, they could be formulated much clearer. The aim of the work, development of a new way of lysozyme modification, is too long and too much in details, what is somehow misleading. The Author should formulate one general objective of the study, followed by a few more detailed and specified aims or goals.

Results and discussion – The scientific content of all four presented papers is related to the effects of microwave and microwave plus oxidation modifications of lysozyme powder (paper 2 and 3) and liquid concentrate (paper 4 and 5) on its oligomerization level, selected functionality (hydrophobicity and solubility) and hydrolytic activity. **Publication 2** (Thermal modification of hen egg white lysozyme using microwave treatment, *Acta Scientarum Polonorum Technologia Alimentaria*, 2020, 19(2), 149-157) aimed at application of microwave radiation to modify hen egg white lysozyme. This preliminary study proved that lysozyme (5%) is able to form oligomeric forms, mostly dimers, with enhanced hydrophobicity but decreased solubility and hydrolytic activity under microwave power between 270 W and 630 W at various pH conditions (4.0, 6.0 and 8.0). An important statement was provided, that although bioactivity of modified lysozyme against *Micrococcus lysodeikticus* decreased as an effect of new treatment, it was still significantly higher comparing with preparations after simple thermal or thermochemical procedure.

Questions: 1. How and on what assumptions the selected conditions for the first, preliminary experiment (Pub. 2) were chosen? 2. Why in the first experiment only 2 ml of the lysozyme solution (suspension?) was treated with microwave radiation? 3. On which base the methodology for the determination of hydrolytic activity of lysozyme (Parry et. al., 1965) was selected for the study? Is there any significant difference between the selected methodology and spectrophotometric methodology by Leśnierowski (2007), Nakimbugwe et. al. (2006) or Touch et. al. (2004)?

Publication 3 (Changes in selected physicochemical properties of lysozyme modified with a new method using microwave field and oxidation. PLoS ONE, 2019, 14(2): e0213021) presents the results and conclusions of the study on the lysozyme modification by microwave radiation (270 W) followed by the chemical treatment i.e. addition of hydrogen peroxide as an oxidizing agent (0%-4%) and freeze-drying as a preservation methods. The main outcome of this paper relates to the confirmation of the positive effects of used microwave radiation, and as well, addition of oxidizig agent on the formation of lysozyme dimers and trimers expressing higher

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hydrophobicity but decreased lytic activity of the enzyme (still higher than after simple thermal treatment). Mr Yang, as a first author, stated also that an applied new method of lysozyme modification is fast and easy option for its functionality improvement.

Questions: 1. What was the reason for using H_2O_2 after microwave treatment? Could the presence of hydrogen peroxide during microwave treatment damage the enzyme molecule and activity?

The third experimental part of the study presented in the Ph.D. dissertation (**Publication 4**: Microwave modification as an excellent way to produce unique lysozyme with potential for food and human health, *Foods*, 2021, 10,1319) describes the effect of microwave power (270 W) followed by hydrogen peroxide addition (1.5%) on the functionality and bacteriolytic activity of the liquid lysozyme concentrate. Similarly to the studies carried out on powder lysozyme, Author(s) proved that low power microwaving together with low concentration of an oxidizing agent resulted in high content of the formed lysozyme oligoforms with higher hydrophobicity and potentially strong and wider antibacterial activity, nonetheless, decreased lytic activity against Gram positive bacteria after the treatment.

In the last part of the thesis the results of the storage experiment carried out on basic and modified (the same conditions as in P4) liquid lysozyme concentrate are presented (**Publication 5** Unconventional effects of long-term storage of microwave-modified chicken egg white lysozyme preparations, *Scientific Reports*, 2021, 11, 10707). Study aimed at evaluation of the functionality and biological activity of the applied material stored for 6 and 12 months under refrigerated conditions shows significant improvement of the microwaved and oxidized enzyme functionality and reveal new antibacterial capabilities related to the time of storage. The longer storage the higher concentration of lysozyme oligoforms with higher surface hydrophobicity. Despite the loss of lytic activity after protein modification procedure, storage at 4-6°C resulted in greater antibacterial ability towards Gram+ and even some tested Gram- bacteria.

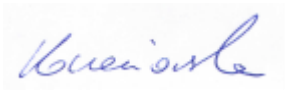
Questions: 1. What microbiological tests can be used to verify the antimicrobial potential of the new form of lysozyme? 2. Has the addition of H_2O_2 been neutralized, if so, on what way? 3. Is the antibacterial effect related to only the lysozyme oligomers or is it due to the presence of hydrogen peroxide? 4. Why other sensory related properties of the preparation were not included in the study, as they could possibly influence color, taste, even flavor of the protein itself affecting final attractiveness of the preparation/food? 5. What are potential applications of the new lysozyme in food industry and beyond? 6. What is the economic efficiency of the new enzyme preparation production?

The main findings of the work are summarized by four conclusions corresponding with the publications included in the PhD thesis. The presented form is a little too much descriptive, rather than strictly and clearly pointing out the most important findings. The good point of the

conclusions part is an indication of future directions in which could be considered in further research on the recognition of the possibility of the new form of lysozyme biological and technological characterization and as well industrial applications.

Final conclusion

Summing up, I would like to state that, the assessed PhD thesis has a scientific potential with novel aspects of lysozyme modifications by physical and physico-chemical methods. All experiments were carefully planned and carried out with properly selected and available classic methodology and equipment. The collected results were presented clearly with new graphical tools (very beneficial and helpful is to apply graphical abstract at the beginning of each experimental part of the thesis), analyzed, discussed and interpreted in a thorough manner. The results were published in the renowned high ranked journals, which prove the novelty and high level of the performed research (Total number of MEiN=550). The content of the doctoral dissertation of MSc. Tianyu Yang entitled: "Identification and physicochemical evaluation of lysozyme after its thermo-oxidative modification with microwave radiation" and the resulting valuable practical application possibilities give me the right to conclude that it meets all the requirements specified in Regulation of the Minister of Science and Higher Education (Art. 261., January 19, 2018) on academic degrees and academic title as well as degrees and title in the field of art (Journal of Laws No. 261, as amended). Therefore, with full conviction, I submit an application to the Council of the discipline Food Technology and Nutrition at the University of Life Sciences in Poznan for the admission of Mr. Tianyu Yang to the public defense of this thesis.



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