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Identification and physicochemical evaluation of lysozyme after its thermo-oxidative modification with microwave radiation

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Abstract

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In nature lysozyme is a protective protein molecule that is found in most living organisms. Its biological function is to destroy Gram-positive bacteria through a mechanism affecting β - 1,4 glycosidic bonds on the cell membrane. These highly efficient antibacterial and nontoxicity characteristics provide lysozyme with good application value in the food industry,medicine and veterinary. However, Gram-negative bacteria are not sensitive to native lysozyme, because the outer membrane structure of bacteria limits the activity of lysozyme acting on it. Several methods are currently known to increase the antimicrobial effectiveness of the enzyme, but new methods are still being sought to unleash its full potential. Chemical and physicochemical modification of lysozyme are an effective direction that can improve this potential by changing the molecular structure of this enzyme, which will greatly improve its practical applicability.

The aim of this doctoral dissertation was to use microwave radiation in combination with oxidation, as well as use a new - liquid form of the raw material to develop a novel original method of lysozyme modification and to evaluate the physicochemical properties of the preparations obtained with this method, as well as demonstrate the extension of the spectrum of antimicrobial activity of such modified lysozyme.

The research work was carried out in stages, and the effects of each of them were presented in a separate publication, which together with the review work constitute a homogeneous series of articles that make up this doctoral dissertation. This series includes the following five publications:

(1) Leśnierowski G., Yang T. 2021. Lysozyme and its modified forms: a critical appraisal of selected properties and potential. Trends in Food Science & Technology, 107, pp. 333-342. https://doi.org/10.1016/j.tifs.2020.11.004

($IF_{2020-21}$: 12.563, MES/MEiN points: 200)

(2) Yang, T., Leśnierowski, G. 2020. Thermal modification of hen egg white lysozyme using microwave treatment. Acta Sci. Pol. Technol. Aliment., 19(2), pp. 149–157. http://dx.doi.org/10.17306/J.AFS.2020.0773

(MES/MEiN points: 40)

(3) Yang, T., Leśnierowski, G. 2019. Changes in selected physicochemical properties of lysozyme modified with a new method using microwave field and oxidation. PLOS ONE 14(2): e0213021. https://doi.org/10.1371/journal.pone.0213021
(IF_{2019}: 2.766, MES /MEiN points: 100)

(4) Yang, T., Leśnierowski, G. 2021. Microwave modification as an excellent way to produce unique lysozyme with potential for food and human health. *Foods, 10(6),* pp. 1319. https://doi.org/10.3390/foods10061319
($IF_{2020-21}$: 4.350, MES/MEiN points: 70)

(5) Leśnierowski, G., Yang, T., Cegielska-Radziejewska, R. 2021. Unconventional effects of long-term storage of microwave-modified chicken egg white lysozyme preparations. Scientific Reports 11, 10707. https://doi.org/10.1038/s41598-021-89849-2
($IF_{2020-21}$: 4.379, MES /MEiN points: 140)

The first one is an introduction to the research, the next three publications present the results of the basic stages of research including the development of the microwave modification of the enzyme and the assessment of the physicochemical properties of the obtained preparations, while the last one presents unconventional changes that took place in lysozyme modified by the microwave method after its long-term refrigerated storage.

Publication (1) provided basic information on lysozyme. It presented a literature review on the characteristics of the enzyme, its structure, sources and production possibilities, modification methods, native and post-modification properties as well as application potential.

The first stage of research involved evaluating the effect and characteristics of microwave radiation as a new energy source for thermal modification of lysozyme. Selected physicochemical properties of lysozyme before and after the microwave modification were measured. Publication (2) presented the results of this research. Research showed that microwave radiation can effectively promote lysozyme oligomerization and leads to changes in protein properties. The modified preparation was characterized by a large content of dimer, higher hydrophobicity with higher solubility. Meanwhile, this modification method also has many advantages resulting from high efficiency, easy operation and lower costs for actual production and operation. Thus, it has the potential for further development and application.

The second stage of research, the results of which were presented in publication (3), involved further exploration of microwave radiation modification methods. A hybrid method of modifying lysozyme using microwave radiation combined with an enzyme oxidation process was evaluated. The assessment showed that this thermo-chemical method efficiently induces the oligomerization of lysozyme. The modified lysozyme formed a greater amount of dimer

and trimer with changes in biological activity and higher surface hydrophobicity, the maximum 58.9% oligomers obtained at 4% oxidant and pH 8, the maximum 38.5% dimer obtained at 4% oxidant and pH 6.0. According to the previous studies, the modified lysozyme can produce dimer-form enzymes with different physical and chemical properties, while these changes lead to an increase of the antibacterial effect of lysozyme. The results of this phase mean that the improved modification method results in a further increase in the overall antibacterial activity of lysozyme.

Publication (4) presented the third stage of research, which included optimization of the microwave enzyme modification conditions. The degree of modification in the acidic environment and duration of the modification process are the two main modification factors that were investigated. In addition, in these studies an innovation was connected with the use of liquid lysozyme concentrate (LLC) as a raw material instead of the previously commonly used lysozyme in the form of dry lysozyme powder. The results showed that the modified lysozyme has significantly improved dimer content and surface hydrophobicity properties, which enhanced the antibacterial activity of lysozyme and the enzyme has the advantage of being completely soluble. Therefore, the use of microwave radiation to modify lysozyme could be simple and fast to produce a large number of high-quality products, which have application potential and attractiveness in many fields, such as food and medicine.

Publication (5) presented the results of additional, previously unplanned studies, which were caused by the observed very interesting changes in microwave-modified lysozyme during its long-term refrigerated storage. It turned out that the modification process throughout such storage was still ongoing, which resulted in an increase in the potential of previously microwave-modified lysozyme. It was shown that the quality of the storage-modified lysozyme increased significantly. The first visible change was a color change to a clearly darker one, which may indicate significant changes in the structure of the enzyme. These changes also affected other physicochemical properties as well as the antibacterial activity of the preparations. The results showed that a significantly increased number of oligomers and hydrophobicity of microwave-modified preparation appeared in the long-term storage process. Especially after 12 months of storage the maximum obtained content of oligomer increases up to 66.4 %, while the maximum hydrophobicity increases up to 72%. The microwave-modified lysozyme significantly enhanced the inhibitory effect on both Gram-positive and Gram-negative bacteria. These unconventional changes during the long-term storage demonstrated that using microwave radiation to modify LLC is an efficient method to obtain high-quality preparation, which has a great practical application potential.

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