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Physicochemical evaluation of preparations obtained as a result of enzymatic modification of lysozyme with pepsin and trypsin†

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Abstract: Lysozyme is a 14.3 kDa protein consisting of 129 amino acids. Modifications of this molecule lead to oligomers and dimers, but more and more attempts are made to break down the lysozyme monomer into smaller molecules. The peptides obtained as a result of these processes can have bioactive properties, thanks to which they can be used in the food, pharmaceutical and medical industries. The aim of the research was to develop a method for the preparation and analytical evaluation of bioactive lysozyme derivatives resulting from enzymatic hydrolytic catalysis of native lysozyme derived from chicken egg white. The factors differentiating the hydrolysis variants were: enzymes (pepsin and trypsin), pH of the mixture (2, 4, 6) and temperature (40, 55 and 70 ° C). The conditions for carrying out the lysozyme modification had a significant impact on the electrophoretic separation, as well as on the hydrolytic, hydrophobic and antioxidant activity of the obtained preparations. The highest percentage of peptides was obtained by hydrolysis with pepsin at the temperature of 70 ° C and pH 4. The obtained preparations obtained as a result of the modification are characterized by significantly higher ($p < 0.05$) antioxidant and hydrolytic activity compared to the lysozyme monomer.

Keywords: lysozyme; bioactive peptides; enzymatic hydrolysis; hydrolytic activity; hydrophobic activity; antioxidant activity

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1. Introduction

Lysozyme obtained from a hen egg has many properties, which makes it the subject of many scientific studies. Due to its hydrolytic activity against microbial cell walls, lysozyme is used in the food industry primarily as a preservative, e.g. in sausages, meat or fish [1,2]. Naturally, lysozyme occurs in the form of a monomer, but under the influence of certain environmental conditions, it can form dimers or oligomers, which can lead to an increase in its antimicrobial properties [3]. In the conducted research it was assumed that the hydrolysis can also obtain changes in the structural structure of the lysozyme molecule (formation of peptides, free amino acids, oligomeric forms) resulting in the opening of the active center and increased hydrophobicity of the enzyme surface. The use of electrophoretic separation as well as densitometric analysis allowed to obtain an answer to the question to what extent and whether lysozyme undergoes thermal-enzymatic hydrolysis carried out in the range of 3 different temperatures and with the use of 2 different types of digestive enzymes. The basic physiochemical properties of the obtained preparations were also assessed, such as: hydrophobic, hydroolytic and antioxidant activity. The aim of the research was to develop a method for the preparation and analytical

evaluation of bioactive lysozyme derivatives resulting from enzymatic hydrolytic catalysis of native lysozyme derived from chicken egg white.

2. Materials and Methods

The modification was carried according to the modified method Carillo W. et al. 2014 [4]. The test material was a 3% aqueous solution of commercially available native lysozyme from Belovo (Belgium). The hydrolytic catalysis was carried out with the use of specifically selected proteolytic enzymes, ie trypsin and pepsin. The hydrolysis processes were run for 60 minutes in the Syncore Analyst analytical reactor by Büchi (Switzerland) and the chemical reactor by Eppendorf Thermo Mixer (Germany). The factors differentiating the hydrolysis variants were: pH of the mixture (2, 4, 6) and temperature (40, 55 and 70 ° C). The hydrolysis reactions were stopped by heating the mixture to 80 ° C for a period of 5 min.The effectiveness of the process conditions was assessed by electrophoresis and densitometry. The next stage of the research was to evaluate the hydrolytic, hydrophobic and antioxidant activity of the preparations [1,3].

3. Results and Discussion

The conditions for carrying out the lysozyme modification had a significant impact on the electrophoretic separation, as well as on the hydrolytic, hydrophobic and antioxidant activity of the obtained preparations. The highest percentage of peptides was obtained by hydrolysis with pepsin at the temperature of 70 ° C and pH 4. The obtained preparations obtained as a result of the modification are characterized by significantly higher ($p < 0.05$) antioxidant and hydrolytic activity compared to the lysozyme monomer. In the studies conducted so far, pepsin has been used much more often. In the work by Carillo et al. 2016 [5] it was shown that in a medium with a pH of 2.0 and using pepsin as a hydrolyzing agent, lysozyme was only partially hydrolyzed. Modification under the same conditions was carried out by the same author also 2 years later, and the results also indicated partial hydrolysis of lysozyme, which led to the release of 23 biologically active peptides [6]. The results obtained in the above-mentioned studies are in line with those obtained in this paper. In the case of trypsin, so far no studies have been conducted in which this enzyme would be used as the only hydrolyzing agent in the lysozyme modification process. The results obtained in this study, indicating a slight but possible degree of lysozyme hydrolysis with trypsin only, encourage the continuation of the research in this area. Therefore, it is reasonable to try to carry out thermal-enzymatic hydrolysis of lysozyme in the presence of trypsin to check whether other environmental conditions will result in a better result than the one obtained in this thesis. Literature data most often indicate the combination of trypsin with e.g. pepsin or papain for the purposes of conducted experiments [7,8].

4. Conclusions

The modification of lysozyme made it possible to obtain preparations with hydrolytic, hydrophobic and antioxidant activity. The conditions for carrying out the lysozyme modification had a significant influence on the electrophoretic separation, as well as on the hydrolytic, hydrophobic and antioxidant activity of the obtained lysozyme preparations. The enzymatic hydrolysis of lysozyme worked best with the enzyme pepsin at 70 ° C and pH 4. The applied modification conditions reduce significantly ($p<0,05$) the hydrolytic activity and increase the antioxidant activity of the obtained preparations in relation to the lysozyme monomer. The temperature of 70 ° C and the use of pepsin in the modification of the lysozyme monomer increases significantly ($p<0,05$) the hydrophobic activity of the obtained peptides. The same modification temperature, but the use of trypsin lowers this activity on the lysozyme monomer.

Conflicts of Interest: The authors declare no conflict of interest.

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