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Proceeding Paper

Physicochemical Evaluation of Preparations Obtained as a Result of Enzymatic Modification of Lysozymes with Pepsin and Trypsin †

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Abstract: A lysozyme is a 14.3 kDa protein consisting of 129 amino acids. The modification of these molecules leads to oligomers and dimers, but more and more attempts are being made to break down lysozyme monomers 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 this research was to develop a method for the preparation and analytical evaluation of bioactive lysozyme derivatives resulting from the enzymatic hydrolytic catalysis of native lysozymes derived from chicken egg white. The factors differentiating the hydrolysis variants were enzymes (pepsin and trypsin), the pH of the mixture (2, 4, 6), and temperature (40, 55 and 70 °C). The conditions for carrying out lysozyme modification had a significant impact on 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 at 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 monomers.

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

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1. Introduction

Lysozymes obtained from hen eggs have many properties, which have made them the subject of many scientific studies. Due to their hydrolytic activity against microbial cell walls, lysozymes are primarily used in the food industry as preservatives, e.g., in sausages, meat, or fish [1,2]. Naturally, lysozymes occur in the form of monomers, but under the influence of certain environmental conditions, they can form dimers or oligomers, which can lead to an increase in their antimicrobial properties [3]. In the conducted research, it was assumed that hydrolysis can also result in changes in the structural structure of lysozyme molecules (formation of peptides, free amino acids, oligomeric forms), resulting in the opening of the active center and increasing the hydrophobicity of the enzyme surface. The use of electrophoretic separation as well as densitometric analysis allowed us to obtain an answer to the question of to what extent and whether lysozymes undergo thermal–enzymatic hydrolysis in the range of three different temperatures and using two different types of digestive enzymes. The basic physiochemical properties of the obtained preparations were also assessed, such as the hydrophobic, hydrolytic, 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 the enzymatic hydrolytic catalysis of native lysozymes derived from chicken egg white.

2. Materials and Methods

The modification was carried according to the modified method of Carillo W. et al., 2014 [4]. The test material was a 3% aqueous solution of commercially available native lysozymes from Belovo (Belgium). The hydrolytic catalysis was carried out with the use of specifically selected proteolytic enzymes, i.e., trypsin and pepsin. The hydrolysis processes were run for 60 min in a Syncore Analyst analytical reactor from Büchi (Switzerland) and a chemical reactor from Eppendorf Thermo Mixer (Germany). The factors differentiating the hydrolysis variants were the pH of the mixture (2, 4, 6) and the 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 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 at pH 4. The 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, lysozymes were only partially hydrolyzed. Modification under the same conditions was carried out by the same author 2 years later, and the results also indicated partial hydrolysis of the lysozymes, 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, no studies have been conducted so far in which this enzyme is used as the only hydrolyzing agent in the lysozyme modification process. The results obtained in this study indicate a slight but possible degree of lysozyme hydrolysis with trypsin only and encourage the continuation of the research in this area. Therefore, it is reasonable to try to carry out thermal–enzymatic hydrolysis of lysozymes 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 conducting experiments [7,8].

4. Conclusions

The modification of lysozymes made it possible to obtain preparations with hydrolytic, hydrophobic, and antioxidant activity. The conditions for carrying out 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 lysozymes worked the best with the enzyme pepsin at 70 °C and at pH 4. The applied modification conditions significantly reduced ($p < 0.05$) the hydrolytic activity and increased the antioxidant activity of the obtained preparations in relation to the lysozyme monomers. The temperature of 70 °C and the use of pepsin in the modification of the lysozyme monomers significantly increased ($p < 0.05$) the hydrophobic activity of the obtained peptides. The same modification temperature but the use of trypsin lowered this activity on the lysozyme monomers.

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