

Research progress in the application of lysozyme in food and medicine field

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Abstract. As an important physiological active factor in the body, lysozyme has a variety of active functions, such as bacteriostasis, antiviral and immunity enhancement. This paper describes in detail the application of lysozyme in food processing fields such as storing meats, prevention of deterioration of alcoholic drinks, fruits and vegetables, and in the treatment of microbial infectious diseases, cancer, virus infection and other medical fields. At the same time, the application prospect of lysozyme combined with new technologies such as chemical modification, immobilization and nanomaterials to form composite antibacterial materials was pointed out.

1 INTRODUCTION

Lysozyme, also known as muramidase or N-acetylmuramide glycanohydrase, is an alkaline enzyme used to hydrolyze the cell wall of microorganisms, which mainly decomposes the β -1,4 glycosidic bonds between N-acetylglucosamine and N-acetylmuramic acid in the cell wall, so that insoluble mucopolysaccharides are decomposed into soluble glycopeptides, leading to the rupture of the cell wall and the lysis of bacteria^[1].

Lysozyme has a wide range of sources and exists in various tissues of the human body. It is distributed in liquids and microorganisms such as egg white of birds and poultry, mammalian tears, milk and plasma, among which egg white is the most abundant. As an important non-specific immune factor in the body, lysozyme has physiological effects such as bacteriostasis and anti-inflammation, enhancement of immunity, treatment of cancer and antiviral. At present, it is mainly used in many fields such as food, medicine, health care products and cosmetics, and it will have great development potential in the field of feed additives in the future.

2 APPLICATION IN FOOD PROCESSING FIELD

2.1 application in meats

Adding an appropriate amount of lysozyme to meat product can effectively inhibit the reaction of enzymes in meat, prevent protein degradation, and prolong the shelf life of food. Bai Jian^[2] studied the preservation technology of chilled beef. The results showed that the meat quality of beef treated with lysozyme and vacuum packaging was ruddy, and the TVBN value was within the standard of first-class freshness, which effectively

inhibited decomposition of protein. At the same time, it could improve the water retention and quality of beef, and prolonged the shelf life of the product to 15 days. In addition, the development trend of preservative is to mix a variety of preservatives to form a composite preservative, which not only reduces the addition of a single preservative, but also obtains the ideal fresh-keeping effect. Studies have shown^[3-4] that lysozyme combined with natural preservatives such as Nisin, tea polyphenols and shell polyose to form a composite preservative used in chilled meat, which has a good synergistic preservation effect and can effectively prolong the shelf life of meat. Liu Kunyi et al.^[5] explored the effect of composite coating of lysozyme and lactoferrin on the preservation of yak meat. It was found that the composite coating could effectively inhibit the microbial proliferation of chilled yak meat, and had no significant effect on sensory quality, which was helpful to prolong the shelf life of meat.

2.2 application in aquatic products

Aquatic products have high water content and are rich in nutrients such as protein and unsaturated fatty acids. However, they are easy to cause corruption in the process of processing, storage and transportation, and reduce the sensory quality of food, and even foodborne diseases. Therefore, lysozyme is also widely used in preservation of aquatic products. Table 1 lists the application of lysozyme in the preservation of aquatic products. A large number of studies have shown that the synergistic treatment of lysozyme with Nisin, chitosan and ascorbic acid can effectively inhibit the physicochemical indexes such as TVBN, pH value and TBARS of aquatic products such as *Metapenaeus ensis*, iced pomfret and *paralichthys olivaceus* during storage, significantly delay the proliferation of microorganisms,

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maintain the sensory quality of products, and effectively prolong the shelf life of aquatic products^[6-9]. In addition, lysozyme is also used to prepare edible film, which has a good preservation effect in the application of aquatic products^[10-12]. Qiuying Li et al. found that the edible bilayer membrane of lysozyme based on chitosan and sodium alginate could destroy the permeability and integrity of bacterial cell membrane, thus effectively inhibit the growth of bacteria, which had a good application prospect in fish preservation.

Table 1. Application of lysozyme in the preservation of aquatic products

types of aquatic products	main materials	application effect
metapenaeus ensis ^[6]	lysozyme-ascorbic acid-citric acid	It can effectively inhibit the reproduction of microorganisms, slow down the oxidation rate of prawn fat, reduce the increase of TVB-N and pH value, have a good preservation effect, and prolong the shelf life of prawns to 6-7 days.
iced pomfret ^[7]	lysozyme-chitosan-nisin	It can effectively inhibit the growth of microorganisms, reduce the increase of TBARS, TVBN and total number of bacteria, slow down spoilage and prolong the shelf life of products.
Paralichthys olivaceus ^[8]	lysozyme-cinnamaldehyde	It significantly decreased the increase of TVBN, K value and TCA soluble peptide content, and maintained the texture characteristics of fish fillets, which was better than that of cinnamaldehyde alone.
perch fillets ^[9]	lysozyme-blueberry leaf polyphenols	Effectively inhibit the proliferation of microorganisms, significantly reduce the increase of TVBN, pH and K value, slow down fat oxidation and effectively prolong the shelf life of sea bass.

2.3 application in dairy products

As a non-specific immune factor, lysozyme has the special effect of killing spoilage microorganisms in the intestinal tract, and it can promote the proliferation of bifidobacteria in the intestinal tract of infants and young children. Adding an appropriate amount of lysozyme to infant food helps to promote the formation of micro coagulant milk from milk casein in the intestines and stomach of infants, which is beneficial to the digestion and absorption of infants; lysozyme can improve defense factors such as Y-globulin and factor P, in order to enhance the resistance of infants and young children to virus infection, especially for premature infants to prevent digestive system diseases and gain weight^[13].

In addition, lysozyme can also be applied to liquid milk, especially pasteurized milk, usually adding 300~600 µg/mL before packaging, which can effectively prolong the shelf life of products; because it also has the characteristic of high temperature resistance, it can also

be used in ultra-high temperature instant sterilization milk. Marwa A et al.^[14] studied the effect of lysozyme and Nisin on inhibiting the growth of spoilage bacteria in pasteurized milk. It was found that the synergistic treatment of lysozyme and Nisin could effectively inhibit the growth and reproduction of bacteria, improve the bacteriological safety from post-treatment of pasteurized milk during storage, and prolong the shelf life of products.

2.4 application in liquor and beverages

The addition of lysozyme to wine, beer and other alcoholic beverages can not only inhibit the growth and proliferation of acidogenic bacteria, but also has no effect on the flavor of wine, and is less affected by alcohol clarifier, so it is an ideal preservative for wine. Chen Kai et al.^[15] have shown that lysozyme could effectively inhibit the growth of lactic acid bacteria and other microorganisms in Italian Riesling ice wine, which could improve the protein stability of wine to a certain extent, and thus prolong the shelf life of wine. In addition, lysozyme can also be added to beverages, which has good antibacterial activity and does not affect the flavor of beverages. Lysozyme is a good preservative for beverages prolongs the storage period of beverage products. Sozbilen et al.^[16] showed that the synergistic treatment of lysozyme and Nisin could effectively control the growth of acid-producing bacteria in fermented beverage, without affecting its unique aroma and flavor, and prolonging the shelf life of the product.

2.5 application in other foods

In the preservation of fruits and vegetables, lysozyme can effectively inhibit or kill microorganisms on the surface of fruits and vegetables, and maintain its original flavor and nutritional value, which has a good preservation effect. Li Cuihong et al.^[17] found that lysozyme combined with ice-water mixture could prolong the shelf life of spinach, effectively maintain its sensory quality, and slow down the formation of nitrite and oxalic acid in spinach. In addition, Kerkaert et al.^[18] also found that lysozyme was used as an allergen indicator to detect the allergic protein behavior of fresh-cut vegetables.

Baked foods such as bread and cakes are prone to spoilage because they contain more oil and water. By adding an appropriate amount of lysozyme, the reproduction of microorganisms can be effectively prevented. Ma Rongkun et al.^[19] also found that the complex preservatives of 0.12% lysozyme, 0.08% natamycin, 0.11% tea polyphenols and 0.15% protamine had better preservation effect on bread and could prolong the shelf life of bread to 17 days.

3 APPLICATION IN MEDICINE FIELD

As a non-specific immune factor of human body, lysozyme can not only effectively kill the invasion of harmful microorganisms, but also play a synergistic role

in the immune system. Therefore, lysozyme can be used in the treatment of infectious diseases such as diabetic nephropathy, bacterial and fungal, and malignant tumors. In addition, scholars have found that lysozyme has application prospects in viral inhibition such as COVID-19.

3.1 application in the treatment of diabetic nephropathy

Diabetic Nephropathy is one of the most common complications of diabetes. At present, it is recognized that advanced glycation end products (AGEs) are the key pathogenic factor leading to renal dysfunction in patients with diabetes. Although traditional chemical drugs can inhibit the production of AGEs, it can not be eliminated effectively, and the side effects of chemical drugs are more likely to aggravate the development of the disease. Cocchietto et al.^[20] studied the effect of lysozyme microcapsules on diabetic rats. The results showed that oral administration of lysozyme microcapsules could significantly remove AGEs in rats serum and its deposition in kidney, prevent glomerular hypertrophy, prevent the production of microalbuminuria, and play an important role in delaying the development of diabetic nephropathy in rats.

3.2 application in the treatment of infectious diseases such as bacteria and fungal

Lysozyme has a wide antibacterial spectrum, which has bacteriostatic effect mainly by destroying the cell wall structure of bacteria, causing contents to escape, and has inhibitory effects on pathogenic microorganisms such as G⁺ bacteria, G⁻ bacteria and fungi. It has been widely used in the treatment of infectious diseases such as oral cavity, skin and upper respiratory tract. Ding Chunyan et al.^[21] studied that lysozyme enteric-coated tablets combined with Kouyanqing grains in the treatment of recurrent oral ulcer could significantly improve clinical symptoms, but also improve immune ability, shorten the course of disease, which had good clinical application value. KemalÇetin et al.^[22] showed that microgels loaded with lysozyme have inhibitory effects on *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, showing a wide range of antibacterial activities, which provided great potential for various applications in biomedicine.

3.3 application in the treatment of malignant tumor

Lysozyme has a synergistic effect on the immune system in the body, and can improve the immunogenicity of tumor cells, so it has the effect of treating malignant tumors. Wang Lijuan et al.^[23] showed that lysozyme combined with Cisplatin could effectively inhibit the proliferation of SMMC-7721 cells and promote the apoptosis of cancer cells, the highest apoptosis rate was 32.5%, and had almost no effect on normal hepatocyte LO2. The main mechanism may be closely related to

lysozyme combined with Cisplatin blocking the expression of PCNA and bcl-2. In addition, lysozyme can also exert anti-tumor effect through anti-angiogenesis. Wang Zhenhua et al.^[24] found that marine low-temperature lysozyme (MLTL) could inhibit the growth of umbilical vein endothelial cells in a dose-dependent manner and prevent angiogenesis, indicating that MLTL may be used as an anti-angiogenic agent in the clinical treatment of cancer.

3.4 application in the treatment of viral diseases

Lysozyme has a positive charge in human body, which can directly react with viral proteins with negative charges, and form complexes with apoprotein, DNA and RNA to make the virus lose its activity, thus playing an antiviral role. Michiko et al.^[25] showed that heat-denatured lysozyme could effectively inactivate mouse norovirus strain 1 (MNV-1). It was found that before heat-denatured lysozyme, adjusting pH to 6.5 or above could enhance the antiviral effect of lysozyme. The mechanism may be that the hydrophobic amino acid of lysozyme, especially residue 5-39, reacted with structural protein of MNV-1 after exposure to thermal denatured, resulting in inactivity of MNV-1, thereby exerting the antiviral effect. In addition, Kelly Mann et al.^[26] found that lysozyme could significantly inhibit the production of proinflammatory cytokines such as TNF- α and IL-6, block the formation of AGEs, and enhance the excretion capacity of kidney. Moreover, when simulated gastrointestinal digestion, hen egg white lysozyme could exhibit significant antioxidant activity and angiotensin converting enzyme (ACE) inhibitory activity, so lysozyme had the potential as an antiviral and immunomodulatory drugs in the treatment of COVID-19.

4 CONCLUSION

In summary, lysozyme has a wide range of applications in various fields such as food processing and pharmaceutical industries. Because of its good antibacterial activity, it is an ideal natural preservative, which can prevent the deterioration of food, improve the nutritional value and prolong the shelf life of products. And it has the application prospects of treating diabetes, cancer and inhibiting COVID-19 and other viruses, so it is widely favored in the field of medicine. In recent years, scientists have made certain progress in the research of lysozyme. Physical modification, chemical modification and nanomaterials technology can not only effectively improve the biological activity and antibacterial efficiency of lysozyme, but also expand the application range of lysozyme. Lysozyme is combined with polymer materials to form a controlled-release antibacterial film, which is applied to the surface coating or food packaging of food. It has efficient antibacterial effect and significantly improves the utilization of lysozyme. In addition, the immobilization of lysozyme based on chemical modification can effectively adsorb bacterial endotoxin, which can be used to develop new medical materials for vitro treatment. With the further study of

lysozyme, it is believed that lysozyme will have a broader application prospect and have a more positive impact on people's lives in the future.

References

1. Fan Linlin, Lin Nan, et al. Research advances on lysozyme and its application in food industry[J]. Food and Fermentation Industries, 2015, 41(03):248-253.
2. Bai Jian. New research on the fresh-keeping technology of chilled meat[J]. Meat Research, 2006(07):43-45.
3. Ou Xiaoqian, Yang Shaohua, et al. Optimization of compound preservative by orthogonal test and its effect on the quality of chilled chicken[J]. Journal of Hefei University of Technology, 2020, 43(08):1136-1142.
4. Dong Wenli, Gong Xue, et al. Quality Change of Chilled Beef under the action of Compound biological Preservative[J]. Packaging Engineering, 2020, 41(03):13-20.
5. Liu Kunyi, Wang Qi, et al. Application of Lysozyme and Lactoferrin in Coating Preservation of Chilled Yak Beef[J]. The Food Industry, 2020, 41(01):100-105.
6. Zhuang Qiuli, Lin Jiancheng, et al. Preservation Effect of Complex Lysozyme Preservative on *Metapenaeus ensis*[J]. The Food Industry, 2019, 40(05):37-41.
7. Zhang Jingjing, Tang Jinsong, et al. The combined use of lysozyme, nisin and chitosan for the preservation of iced pomfret[J]. Science and Technology of Food Industry, 2014, 35(04):323-326.
8. Yongxia Xu, Yiming Yin, et al. Effects of lysozyme combined with cinnamaldehyde on storage quality of olive flounder fillets[J]. Journal of Food Science, 2020, 85(4):1037-1044.
9. Lin Zhiming, Wang Yuanyuan, et al. Effect of Blueberry Leaf Polyphenols and Lysozyme on Storage Quality of Perch Fillets[J]. Farm Products Processing, 2018(21):36-39+44.
10. Qiuying Li, Jinxiu Xu, et al. Preparation of a bilayer edible film incorporated with lysozyme and its effect on fish spoilage bacteria[J]. Journal of Food Safety, 2020, 40(5).
11. Tiantian Wu, Yujun Ge, et al. Quality enhancement of large yellow croaker treated with edible coatings based on chitosan and lysozyme[J]. International Journal of Biological Macromolecules, 2018.
12. Zhang Jiatao, Zhang Xuan, et al. Preservative Properties of TP Microcapsules/LZM-PVA Composite Coatings on *Sciaenops ocellatus* Fillets[J]. Science and Technology of Food Industry, 2020, 41(08):273-278+284.
13. Hao Changming, Qian Fang, et al. Academic Proceedings of the 14th China International Food Additives and Ingredients Exhibition[C]. China Food Additives Magazine, 2010:4.
14. Saad Marwa A, Ombarak Rabee Alhossiny, et al. Effect of nisin and lysozyme on bacteriological and sensorial quality of pasteurized milk[J]. Journal of advanced veterinary and animal research, 2019, 6(3):403-408.
15. Chen Kai, Han Shun-Yu, et al. Development of lysozyme-combined antibacterial system to reduce sulfur dioxide and to stabilize Italian Riesling ice wine during aging process[J]. Food science & nutrition, 2015, 3(5):453-465.
16. Gozde Seval Sozbilen, Figen Korel, et al. Control of lactic acid bacteria in fermented beverages using lysozyme and nisin: test of traditional beverage boza as a model food system[J]. International Journal of Food Science & Technology, 2018, 53(10):2357-2368.
17. Li Cuihong, Wei Lijuan, et al. Effect of Combined Treatment with Ice Water and Lysozyme on Spinach Quality and Nitrate Content[J]. Food Science, 2020, 41(07):203-209.
18. Barbara Kerkaert, Liesbeth Jacxsens, et al. Use of lysozyme as an indicator of protein cross-contact in fresh-cut vegetables via wash waters[J]. Food Research International, 2012, 45(1):39-44.
19. Ma Rongkun, Hou Dongli, et al. Application of Natural Preservatives in Bread Preservation[J]. The Food Industry, 2019, 40(06):114-117.
20. M. Cocchietto, L. Zorzini, B. et al. Orally administered microencapsulated lysozyme downregulates serum AGE and reduces the severity of early-stage diabetic nephropathy[J]. Diabetes and Metabolism, 2008, 34(6):587-594.
21. Ding Chunyan, Zhang Jianquan. Clinical study on Kouyanqing grains combined with lysozyme Enteric-coated Tablets in treatment of recurrent oral ulcer[J]. Drugs & Clinic, 2019, 34(05):1426-1429.
22. Kemal Çetin, Sevgi Aslıyüce, et al. Preparation of lysozyme loaded gelatin microcryogels and investigation of their antibacterial properties[J]. Journal of Biomaterials Science Polymer Edition, 2020(7):1-13.
23. Wang Lijuan, Li Linna, et al. Synergetic inhibition effects of lysozyme and cisplatin against proliferation of human liver cancer SMMC-7721 cells[J]. Journal of Jinan University, 2008(02):130-133+142.
24. Zhenhua Wang, Jincheng Liu, et al. Antiangiogenic activity of low-temperature lysozyme from a marine bacterium in vivo and in vitro[J]. Chinese Journal of Oceanology and Limnology, 2009, 27(4):835-844.
25. Takahashi Michiko, Takahashi Hajime, et al. Impact of pH and protein hydrophobicity on norovirus inactivation by heat-denatured lysozyme[J]. PLoS one, 2020, 15(8).
26. Jaclyn Kelly Mann, Thumbi Ndung'u. The potential of lactoferrin, ovotransferrin and lysozyme as antiviral and immune-modulating agents in COVID-

19[J]. Future Virol, 2020.