Preparation of antimicrobial peptides from Bacillus subtilis and effect on fresh-cut vegetables

Zhou Miao¹, Chang Xiguang², Feng Xiaoguang², Ren Yi¹, Chen Chichang¹, Chen Xiangning¹*

¹Food Science and Engineering College, Beijing University of Agriculture, Beijing 102206, China;
²Beijing Yunong High Quality Cultivation of Agricultural Products Company, Beijing 101400, China

*Corresponding author’s e-mail: cxn@bua.edu.cn

Abstract: In order to extend the shelf life of fresh-cut vegetables, the principle of preservation of Bacillus subtilis antimicrobial peptides was discussed. Fresh potatoes, yam and lotus root were used as test materials, and the prepared Bacillus subtilis antimicrobial peptides were used for soaking treatment, soaked in clean water as a control, and stored at 4°C. The sensory quality changes during storage were analyzed and their physiological and biochemical indexes were determined. The results show: compared with the control group, the group treated with Bacillus subtilis antimicrobial peptides had the best sensory quality during storage, significantly inhibited the increase in electrical conductivity, reduced cell membrane damage, and had significant POD and PPO enzyme activities (P<0.05). The reduction effectively inhibits the proliferation of microorganisms during the storage of fresh-cut vegetables, thereby better maintaining the quality of fresh-cut vegetables and effectively extending their shelf life.

1. Introduction

Compared with intact vegetables, fresh-cut vegetables are prone to texture softening, physiological aging, tissue browning, nutrient loss, excessive microbes, and decreased flavor due to damage to the tissue structure, and it is difficult to maintain their excellent quality. The quality greatly shortens the shelf life and limits the development of fresh-cut vegetables. Therefore, research on the problems that occur during storage of fresh-cut products is of great significance.

At present, the preservation of fresh-cut vegetables mainly uses chemical reagents combined with physical techniques such as low temperature and air conditioning for preservation. However, chemical reagents have hidden dangers of drug residues and are easy to cause environmental pollution. Therefore, looking for a safe and effective antistaling agent has become a hot spot in the study of fresh-cut vegetables storage and preservation. Bacillus subtilis (Bacillus subtilis) is a kind of Bacillus. It is widely used in the production of industrial enzymes and chemicals[1]. At present, studies have shown that small molecule polypeptides isolated from liquid fermentation products of Bacillus subtilis has antibacterial activity[2]. After harvested grapes, soaking Bacillus subtilis culture can effectively inhibit the growth of mold[3]. It has the advantages of naturalness, non-toxicity, and no residue. However, there are few reports on the use of Bacillus subtilis antimicrobial peptides to keep fresh-cut vegetables.
Therefore, three commonly used fresh-cut vegetables: potato, yam, and lotus root are selected as test materials, to explore the mechanism of Bacillus subtilis antimicrobial peptides on fresh-cut vegetables, and provide a certain reference value for the development of fresh-cut vegetables.

2. Materials and methods

2.1. Main materials and instruments

2.1.1. Materials
Potato, yam, lotus root, Bei Nong Market; B. subtilis (strain S1702) was isolated in advance from soil and stored in the Food Biotechnology Lab of Beijing Forestry University, Beijing, China.

2.1.2. Main reagents and instruments
PCA agar medium, glucose, peptone, sodium chloride, agar powder, sodium hydroxide, ethanol. All other reagents were of analytical grade.
DGN-06 multifunctional fresh-keeping sealing machine, Ningbo Xiangshan Lvyuan Light Industrial Machinery Manufactory; H1850R desktop high speed refrigerated centrifuge, Xiangyi Centrifuge Instrument Co., Ltd; T6 new century spectrophotometer, Beijing Spectrum Analysis General Instrument Co., Ltd.; BCD-288wsl refrigerator, Qingdao Haier Co., Ltd.

2.2. Experimental methods

2.2.1. Bacillus subtilis antimicrobial peptide(AMP) preparation
Bacillus subtilis strain activation: B. subtilis was activated in NA(glucose 20g, yeast extract 5g, peptone 10g, NaCL 5g, agar 15g dissolved in 1L distilled water, pH 7.0) medium and incubated for 24 h at 37°C. Cell suspension preparation: A loop of activated Bacillus subtilis was inoculated into LB(yeast extract 5g, peptone 10g, NaCL 5g dissolved in 1L distilled water, pH 7.0) liquid medium, incubated at 37 °C for 24 h with shaking.

AMP Preparation: According to the high density culture method of Bacillus subtilis.[5] To obtain a conventional B. subtilis liquid culture (1×10^8cfu/mL), 5 mL of the prepared cell suspension were inoculated in 100 mL conventional medium, AMP was obtained by centrifuging at 10,000 g for 20 min and then filtering through a Millex-HV filter.

2.2.2. Pretreatment of materials
Pick out potatoes, yams, lotus roots of similar color and size. Then wash off the dirt, and cut potato, yam and lotus root into 5 mm thick slices with stainless steel knife after peeling. Cleaning with 100 ppm sodium hypochlorite. Then the experimental group was immersed in AMP solution for 5 min, Control group soaked in water for 5 min, Spin the processed potato, yam, and lotus root slices in a spin dryer. Each experiment is done 3 times in parallel. The samples are stored at 4℃for 9 days. Sensory evaluation, physiological and biochemical indicators and microorganisms are measured on the samples stored for 1, 3, 5, 7 and 9 days.

2.2.3. Determination of sensory indicators
According to the method by Wang Mei[4] et al., the experimental method is slightly changed.

2.2.4. Determination of relative conductivity
It was determined by Li Peiyuan[4] et al. with slight modification.

2.2.5. Determination of polyphenol oxidase(PPO) and peroxidase(POD) activities
PPO activity was determined by catechol method[6]; POD activity was determined by guaiacol method[7].
2.2.6. Determination of total colony count
The detection method of colony count refers to GB 4789.2—2016 determination of colony count in national standard food microbiology of food safety.

2.2.7. Data processing
The above experiments were repeated three times, and the measured data were processed by Excel software.

3. Results and analysis

3.1. Effects of different treatments on sensory quality of fresh-cut vegetables during storage

![Sensory Score vs. Storage Time](image)

Fig1. Effect on sensory quality of different treatment groups during storage
Sensory evaluation is the most intuitive evaluation method for fresh-cut vegetables. After the vegetables are cut, they cause mechanical damage to the tissues and accelerate the changes in color and texture. In this test, a comprehensive score was made from browning area, color, texture, decay degree and smell. As shown in Figure 1, during the 9 d storage period, the sensory evaluation scores of the three fresh-cut vegetables continued to decrease, and the sensory quality decreased more rapidly in the later period of storage. During the entire storage period, the sensory scores of the three vegetables treated with AMP were better than those of the control group at each stage of storage. From the 3rd day of storage, potatoes and lotus roots treated with clean water began to brown on the edges and slightly sour. Potatoes showed slight water soaking, lotus roots began to lose water, and the surface slightly shrank. On the whole, the preservation effect of AMP is better than that of the control group.

3.2. Effects of Different Treatments on Relative Conductivity of Fresh-cut Vegetables During Storage

![Relative Conductivity vs. Storage Time](image)

Fig2. Effect on relative conductivity of different treatment groups during storage
The relative conductivity of fresh-cut vegetables can reflect the degree of damage to the membrane system. When vegetables are processed, the cell membrane of the cut tissue is damaged, which easily
leads to electrolyte leakage, which leads to an increase in conductivity. As shown in Figure 2, the relative conductivity shows an upward trend with the extension of storage time. During the storage period, the treatment group maintained a lower electrical conductivity than the control group, indicating that the treatment with Bacillus subtilis antimicrobial peptides can maintain the integrity of the cell membranes of fresh-cut vegetables during storage; The electrical conductivity of the three kinds of vegetables changed relatively smoothly, possibly because the vegetables gradually lost their physiological activity as the storage time extended. Taken together, AMP can inhibit the increase in relative conductivity of the three fresh-cut vegetables, reduce cell membrane damage, and improve the preservation effect.

3.3. Effects of different treatments on the activities of POD and PPO enzymes in fresh-cut vegetables during storage

POD is a key enzyme that promotes the formation of fresh-cut vegetable callus and enzymatic browning. In the presence of H2O2, POD can rapidly oxidize phenolic substances, causing browning of fresh-cut vegetables, and inhibiting the activity of fresh-cut vegetables POD is to prolong the preservation One of the indispensable conditions for the period. As shown in Figure 3, the POD activity of vegetable slices in different treatment groups showed an overall upward trend during storage, and the POD activity of the AMP treatment group was lower than that of the water treatment group, with significant differences (P<0.05). The results showed that AMP treatment can inhibit the increase of POD activity of three fresh-cut vegetables, and delay callus formation and oxidative browning. PPO is the most important enzyme that promotes the enzymatic browning reaction of fresh-cut vegetables during processing, storage and processing. Therefore, it is very important to inhibit PPO activity during the storage of fresh-cut vegetables. As shown in Figure 4, from the beginning of storage to the 3rd day, the PPO activity of the three dishes showed an upward trend and peaked on the 3rd day. After the 3rd day of storage, the PPO enzyme activity showed a downward trend. The activity gradually tended to 0 U/min·g, and the PPO enzyme activity of the three vegetable treatment groups was significantly lower than that of the control group. This shows that AMP can effectively inhibit the activity of PPO, thereby delaying the browning of fresh-cut vegetable slices.
3.4. Effects of Different Treatments on the Total Colony of Fresh-cut Vegetables During Storage

The fresh-cut vegetables after peeled and cut, the tissues are damaged and nutrients flow out, providing a good environment for microorganisms to multiply. The trend of the total number of colonies is shown in Figure 5. During the entire storage period of the three fresh-cut vegetables, the AMP treatment group had better antibacterial effects than the control group. The total number of colonies of fresh-cut potatoes, yams, and lotus roots treated with AMP were lower than the water treatment group on the 1-3 days of storage. It can be seen that the antimicrobial peptide treatment can significantly inhibit the growth of microorganisms in the early storage period. And until the 5th day of storage, the AMP treatment group The number of microorganisms grows slowly. As the storage time increases, the number of microorganisms gradually increases. After the 7th day of storage, the number of microorganisms in all groups begins to increase significantly. It can be judged that the antimicrobial peptides gradually lose their antibacterial activity after the 5th day active. The results showed that AMP had a significant antibacterial effect in the early stage of storage, and had a better effect on fresh-cut vegetables.

4. Conclusion

Three kinds of vegetables were selected in this experiment: potato, yam, lotus root, as the test materials, treated with AMP as the experimental group, soaked in clear water as the control group, stored at 4℃ for 9 days, and analyzed the sensory quality changes of fresh-cut vegetables during storage. Physiological and biochemical indicators and the total number of colonies were measured. The results showed that the AMP treatment group can reduce the relative conductivity of the three fresh-cut vegetables during storage, reduce the degree of cell membrane damage, and keep the PPO and POD enzymes at a low level, effectively inhibiting the proliferation of microorganisms during the storage of fresh-cut vegetables is prevented, the quality of fresh-cut vegetables is delayed, and the shelf life of fresh-cut vegetables is extended by 2-3 days. It provides a certain reference value for finding a natural, non-toxic and harmless preservative.

Acknowledgement

We extend special thanks to the Beijing Innovation Consortium of Agriculture Research System (BAIC07-2020) and Beijing University of Agriculture Collaborative Innovation Team Construction Plan-Fruit and Vegetable Quality and Fresh-keeping Collaborative Innovation Team

References

[1] Kobbi, S., Balti, R., Bougadef, A., Le Flem, G., Firdaous, L., Bigan, M., Nedjar, N. (2015). Antibacterial activity of novel peptides isolated from protein hydrolysates of RuBisCO purified from green juice alfalfa[J]. Journal of Functional Foods, 18:703-713.

[2] Zhang B., Wang J., Ning S., Yuan Q., Chen X., Zhang Y., Fan J. (2018). Peptides derived from
tryptic hydrolysate of Bacillus subtilis culture suppress fungal spoilage of table grapes[J]. Food Chemistry, 239:520-528.

[3] Zhang B., Li Y., Zhang Y., Qiao H., He J., Yuan Q., Chen X., Fan J. (2019). High-cell-density culture enhances the antimicrobial and freshness effects of Bacillus subtilis S1702 on table grapes (Vitis vinifera cv. Kyoho)[J]. Food Chemistry, 286: 541-549.

[4] Wang Mei, Wu Fangjia, Xu Li, Liu Yongxiang, Li Fei, Chen Chao, Lei Zunguo, Liu Jia. (2020). Effects of Lactic Acid Pretreatment on Cold Storage Quality and Physiological Characteristics of Fresh-cut Potatoes[J]. Journal of Chinese Institute of Food Science and Technology, 20(08):207-214.

[5] Li Peiyan, DANG Dongyang, YIN Fei. (2020). Effect of Oxalic Acid on Fresh Cut Yam Storage and Preservation[J]. Journal of Henan Agricultural Sciences, 49(07):168-173.

[6] Jiang, H., Tong, J., & Lin, Q. (2006). Kinetic characteristics of polyphenol oxidase in Agrocybe aegerita. Preservation and processing, 2006, 6(5): 23-24.

[7] Li, H. (2002). Modern plant Physiology. Higher Education Press, 2002, 2:415-420.