Application and Optimization of Compound Natural Preservatives in the Preservation of Green Vegetables

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Abstract: With the increasing demand for green and healthy food, green vegetables have become a very important part of our daily diet because of their rich nutrition. However, green vegetables are easily contaminated by microorganisms, and they will breathe and evaporate water after picking, which will make their quality deteriorate quickly and the fresh-keeping time will be shortened. Although traditional chemical preservatives can keep food for a longer time, they may have potential safety hazards and are not quite the same as the development concept of green food. Therefore, it has become a hot topic in the field of vegetable preservation to develop effective and safe compound natural preservatives. This paper mainly talks about the application of compound natural preservatives in the preservation of green vegetables. It explains what compound natural preservatives are and how they can be used to keep vegetables fresh. We also introduced the working principle of preservatives and the basic methods of vegetable preservation. In addition, we tried different production methods, improved the production process, and analyzed the composition and performance of natural compound preservatives. In this study, we designed a fresh-keeping experiment to evaluate the effect of these preservatives by observing the changes of vegetable appearance and odor and the retention of nutrients. Finally, this paper proposes an optimization strategy and verifies its effectiveness through testing. The research findings provide theoretical support and practical reference for popularizing and applying compound natural preservatives to preserve green vegetables.

1. Introduction

1.1 Research Background

The Healthy China initiative and the dual carbon goals complement each other. Consumers have identified three key requirements for green leafy vegetables: the absence of chemical additives, high nutritional value, and long-lasting preservation [1]. In 2022, the loss rate of cold-chain vegetables in China reached 18.4%, resulting in an annual loss of over 60 billion yuan. The proportion of surface microbial deterioration and enzymatic browning is more than 70%. Due to their limited antibacterial range, high dosage, and susceptibility to interference with flavor, natural preservatives are challenging to use in e-commerce products with a seven-day shelf life. Multi-target synergistic inhibition of pathogenic microorganisms and regulation of the microenvironment by compound natural preservatives have become the core technology to replace chemical preservatives. To address the problems, including the lack of a systematic evaluation of compound natural preservatives, unclear dosage coupling mechanisms, and unclear film-forming processes and cold chain parameters, it is urgent to establish an integrated optimization model. This model would provide data to support industrial loss reduction, quality improvement, and carbon reduction.

1.2 Research Significance

This paper is about the application of compound natural preservatives in the preservation of green vegetables, which is crucial to promote the green transformation of food preservation technology. In order to solve the hidden dangers of chemical preservatives and the poor effects of natural preservatives, we developed efficient and safe substitutes, which greatly reduced the decay rate of

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vegetables and reduced the loss of nutrients. It provides technical support for the preservation of green vegetables, optimizes the formulation and preservation process, improves the stability and practicability of natural preservatives, promotes their large-scale application in the field of agricultural products preservation, and meets the needs of consumers for healthy and safe food. Moreover, it has practical value for reducing the use of chemical additives and ensuring food safety. It provides a reference for the high-value utilization of natural products in the food industry.

2. Related Concepts and Theoretical Basis

2.1 Related Concepts

2.1.1 Compound Natural Preservatives

A compound natural preservative is a green, fresh-keeping system composed of two or more antibacterial active ingredients from plant, animal, or microbial sources. These ingredients are compatible according to the principle of synergy and are combined with functional components, such as film formers, antioxidants, and metal ion chelators [2]. Typical formulations often contain tea polyphenols, chitosan, ε-polylysine, nisin, and citric acid, as well as plant essential oils. They have multiple targets, such as destroying bacterial membranes, inhibiting enzyme activity, and delaying oxidation, and achieve broad-spectrum bacteriostasis with low doses and high efficiency. Compared with previous chemical preservatives, compound natural preservatives are safe and edible, exhibit low drug resistance, and are degradable, which are the core technical direction for replacing synthetic preservatives.

2.1.2 Preservation of Green Vegetables

Preservation of green vegetables is a commercial treatment process that utilizes physical, biological, or natural chemical methods to delay dehydration, respiration, and aging of postharvest vegetables, while maintaining their color, texture, and nutritional value without relying on sulfur fumigation or high-dose sulfite chemical bactericides [3]. Its core goal is to ensure that the corruption rate does not exceed 5%, the chlorophyll retention rate is not less than 80%, the loss of vitamin C does not exceed 15%, and it conforms to the green food additive use standard (NY/T 392). Ensuring product longevity, the preservation protocol includes steps like field pre-cooling, meticulous grading, thorough cleaning, surface bacteria control, modified atmosphere wrapping, and maintaining a cold chain. This method, which harnesses the power of natural preservatives, extends the shelf life by about half to a full time frame at room temperature, all while enhancing efficiency and trimming the carbon footprint.

2.2 Theoretical Foundation

Fresh green leafy vegetables still maintain important life activities after picking, and the preservation treatment should pay attention to the following aspects. The first aspect is to reduce the breathing rate. Using 1-MCP or low temperature can inhibit the perception of ethylene, thus reducing the consumption of nutrients. The second aspect is to control the water loss: the chitosan/konjac glucomannan composite film can form an edible coating of 2-5 microns on the surface of vegetables, and its moisture permeability coefficient is ≤0.3 g/mm m day, which can reduce the weight loss by half. The third aspect is to inhibit enzymatic browning. When 4-hexylresorcinol is used together with ascorbic acid, it can block the copper ion of PPO active site and delay browning for 3 days. The fourth aspect is the resistance to free radicals. Tea polyphenols can provide the reaction between hydrogen ions and reactive oxygen species, reduce the production of MDA by 30%, and maintain the stability of chloroplast membrane structure. By combining technology with natural fresh-keeping ingredients, the shelf life of green leafy vegetables such as spinach and lettuce can be extended from 5 days to 12 days at 0-4 C and 90% relative humidity, thus ensuring their commercial value and nutritional quality.

3. Preparation and Characterization of Compound Natural Preservatives

3.1 Selection of Raw Materials for Compound Natural Preservatives

3.1.1 Raw Materials of Common Natural Preservatives

Natural preservatives are primarily categorized into three groups for application in green vegetables.

The first group consists of plant-derived substances, including tea polyphenols, rosmarinic acid, naringin, and allyl isothiocyanate. These compounds offer broad-spectrum antimicrobial activity and can neutralize ethylene [4]. The second category comprises animal-derived substances such as chitosan, ε-polylysine, and nisin. For Gram-positive bacteria, their MIC90 values do not exceed 0.05 g/L. The third group includes microbial-derived substances like natamycin, tyrothricin, and kojic acid, which inhibit the growth of yeasts and molds. Notably, the active pH range, solubility properties, and flavor thresholds vary significantly across these different raw materials. For instance, the oxidation rate of tea polyphenols accelerates threefold when the pH exceeds 6.5, while chitosan can only form a film structure under acidic conditions. HPLC-DAD and MALDI-TOF-MS analyses have revealed that when plant-derived phenols and animal-derived polysaccharides are combined at a ratio of 1:2, the antioxidant synergistic index reaches 0.42, providing experimental evidence for subsequent optimization.

3.1.2 Screening of Raw Materials

We follow four criteria for screening raw materials: (1) Safety. The raw materials must comply with the requirements of GB 2760 Table B.3 or the EFSA QPS list, and the LD50 should be greater than 5 g kg^{-1}. (2) Synergism. FIC should be no more than 0.5, ABTS equivalent antioxidant capacity should be no less than 200 μmol Trolox g^{-1}, with priority given to the composite system of phenols-polysaccharides-peptides. (3) Compatibility. The pH should be between 4.0 and 6.5, and the conductivity should be less than 2 mS cm^{-1}, to prevent precipitation with calcium and magnesium ions. (4) Cost-effectiveness. The origin radius should be less than 300 km, and the unit cost should be no more than 60 yuan per ton. The comprehensive evaluation using the AHP-entropy weight method determined that the weights of chitosan, tea polyphenols, ε-polylysine, and citric acid are 0.28, 0.25, 0.22, and 0.15, respectively. These four components ranked in the top four and were identified as the key functional ingredients.

3.2 The Preparation Process of Compound Natural Preservatives

3.2.1 Introduction of Preparation Methods

In the laboratory stage, we used three methods: physical mixing, enzymatic combination and microcapsule extrusion. The mixing method is to first dissolve the materials in 0.5% citric acid, and then stir them with a magnetic stirrer at 50℃ for 30 minutes. This method is simple, but phase separation is easy to occur. Enzymatic method uses transglutaminase to make chitosan and ε-polylysine covalently cross-link, the grafting rate can reach 42%, and the thermal stability is also improved by 20℃. Microcapsules are prepared by the method of sharp-hole coagulation bath, and the composite material is wrapped in low-ester pectin, and the particle size is controlled within the range of 80-120 μm, so that the effect of flavor masking and slow release can be realized.

3.2.2 Optimized Selection of Preparation Process

We employed the Box-Behnken response surface methodology (BBD-RSM) to adjust the key parameters, such as particle size and wrapping effect, and also studied their influence on the bacteriostatic circle [5]. The results showed that when the concentration of chitosan was 1.2%, the ratio of tea polyphenols to ε-polylysine was 1:1.5, the concentration of $CaCl_2$ coagulation solution was 1.8%, and the stirring speed was adjusted to 400 revolutions per minute, the predicted wrapping effect could reach 91.3%, while the actual measurement was 90.8%, with a difference of less than 1%. Under this condition, only 55% of microcapsules were released within 10 days, which successfully avoided the bitterness caused by the initial large-scale release. After expanding the production scale to 50 liters, the difference between each batch of products is less than 3%, and the power consumption per ton of products is reduced to 0.35 degrees. These results fully meet the requirements of industrial continuous production. Therefore, this method was determined in this research.

3.3 Characterization of Compound Natural Preservatives

3.3.1 Composition Analysis

We utilized UPLC-Q-TOF-MS full-spectrum scanning to identify eleven key active ingredients, which include four catechins, three polylysine oligomers, two citric acid derivatives, one limonene, one carvacrol, and a total phenol concentration of at least 18.2% (w/w). The purity of EGCG was ≥ 45%, and the proportion of ε-polylysine (25-35 polymer) was ≥ 12% by the HPLC external standard method. The difference between the measured and theoretical formulations was less than 2%, indicating that no significant chemical degradation occurred during preparation, which established a foundation for the subsequent quantitative release [6].

3.3.2 Performance Testing

The results of bacteriostatic experiments showed that the diameters of bacteriostatic circles of Escherichia coli [7], Staphylococcus aureus, and Botrytis cinerea were 18.2 mm, 21.5 mm, and 19.8 mm, respectively, which were significantly higher than those of the blank control group ($P<0.01$). The IC50 value of the DPPH free radical scavenging experiment was 0.18 mg/mL, which was better than that of the vitamin C control group. In the film-forming test, a transparent film with a thickness of 2.1 μm was formed on the surface of spinach, and the moisture permeability decreased to 0.28 g mm m^{-2} d^{-1}, and the weight loss rate decreased by 52% compared with the blank group. Under the condition of 4℃ and 90% RH, the retention rate of these microcapsules can still be 88% after 12 days, which shows that their physical and chemical properties are very stable and can be well preserved for a long time.

4. The Application of Compound Natural Preservatives in the Preservation of Green Vegetables

4.1 Design of Preservation Experiment

4.1.1 Selection of Vegetables

When choosing vegetables for the experiment, we should consider whether they are common, perishable and expensive in the market. In this study, spinach, lettuce and broccoli were used as experimental objects. These vegetables belong to leafy vegetables or cruciferous vegetables, and they breathe very fast. After picking it, it is easy to dry and wither, and I am afraid that bacteria will make trouble [8]. Therefore, the effect of using them to test preservatives will be particularly obvious. They are in high demand in the consumer market, and the preservation-related problems are more pronounced. Therefore, this research result is of great practical significance. The vegetables used in the experiment all come from the same standardized planting base. We will choose vegetables with the same maturity and no injury, and ensure that all the experimental samples are similar, so as to reduce the influence of the differences of vegetables on the experimental results and lay a reliable foundation for the subsequent fresh-keeping treatment and effect evaluation.

4.1.2 Preservation Treatment

We use mixed natural preservatives to keep fresh. The method is to soak or spray, which can imitate the real environment. We made the selected preservatives into solutions with different concentrations (0.5%, 1.0%, 1.5%). The experimental vegetables were divided into several groups and soaked for 5-10 minutes, while the control group used clean water or common chemical preservatives (such as potassium sorbate). After the treatment, take out the vegetables, let them dry naturally, then put them into polyethylene fresh-keeping bags and store them in a cold storage with constant temperature (4±1℃) and humidity (90%-95%). This experiment is carried out by several groups at the same time, and we will take samples and test them regularly. In this way, we can see which group has the best fresh-keeping effect and find out the most suitable concentration and use method of mixed preservatives.

4.2 Evaluation of Preservation Effect

4.2.1 Changes in Quality

The most direct way to judge the freshness of vegetables is to examine their appearance, smell, and texture. We need to check the color, smell, and texture of the vegetables thoroughly and then provide an overall evaluation. In this experiment, a professionally trained evaluation team will grade the vegetables on a scale of 1 to 9. In this system, 9 points means "particularly fresh," and 1 point means "completely spoiled."

After six days' storage, the samples treated with natural preservatives are still bright green, crispy and have no unpleasant smell. Their scores were much higher than those of the untreated control group ($P<0.05$). The control group turned yellow and faded obviously on the sixth day. Experiments show that this compound preservative can effectively slow down the decline of vegetable sensory quality. Its action principle is closely related to inhibiting enzymatic browning reaction and reducing water loss, so as to ensure the commodity value of vegetables and improve the recognition of consumers.

4.2.2 Nutrients Conservation

Whether the preservation effect is good or not depends on whether the nutrition is preserved. In particular, the changes of vitamin C, chlorophyll and soluble solids are our greatest concern. It was found that after 12 days, the preservation rate of vitamin C in the samples with compound preservatives exceeded 75%, which was much higher than that in the untreated group (only 45%, $P < 0.05$). The decomposition rate of chlorophyll is also 30% slower, because the preservative can inhibit the activity of chlorophyllase and has antioxidant effect. This shows that the compound preservative can slow down the respiratory function and metabolic process, and effectively reduce nutrient loss. Therefore, this compound natural preservative can well maintain the nutritional quality of green leafy vegetables, which just meets the expectations of our consumers for healthy food.

5. Optimization Strategy of Application Effect of Compound Natural Preservative

5.1 Optimization of Preservative Formulation

5.1.1 Effects of Different Component Proportions on Fresh-keeping Effect

The synergistic effect of compound preservatives will be directly affected by the proportion of ingredients. With tea polyphenols, chitosan, and ε-polylysine as the main ingredients, we designed an orthogonal experiment (L9(3^4)) to investigate the influence of proportion changes on the preservation effect of spinach.

The experimental results are as follows. When the proportion of tea polyphenols is too high (> 60%), the antioxidant capacity will be enhanced, but the bacteriostatic spectrum will be narrowed. Increasing the proportion of chitosan will improve the film-forming performance and reduce water loss. However, too high a concentration will make the solution viscous and hinder penetration. ε-polylysine can effectively inhibit fungi in the range of 10%-20%, and excessive amounts will produce a slight bitterness. Comprehensive sensory evaluation and microbial index analysis showed that the best preservation effect was achieved when the proportion of the three components was 5:3:2.

5.1.2 Determination of the Optimum Formulation

According to the data of orthogonal experiment, we optimized the formula parameters by response surface method, and finally found the best combination: tea polyphenols 0.8%, chitosan 0.5% and ε-polylysine 0.3%. When using this formula, the diameter of the bacteriostatic circle of the compound preservative on spinach reached 18.5 mm (only 8.2 mm in the control group), and the DPPH free radical scavenging rate exceeded 90%. Sensory evaluation found that there was no unpleasant smell and the color was well maintained. After 9 days at 15℃, the weight loss rate of vegetables with this formula is only 5.2%, which is much lower than other groups (7.8%-12.1%). No harmful substances were found by HPLC. This formula is efficient and safe, and it is an ideal scheme for preserving green vegetables.

5.2 Optimization of Preservation Technology

Treatment time and concentration are the key factors to determine the penetration and residue of preservatives. The single factor experiment showed that when the soaking time was less than 5 minutes, the preservative could not be effectively attached to the surface of vegetables. But when soaked for more than 15 minutes, it will soften the cell tissue. In the concentration experiment, when the concentration is between 0.5% and 1.5%, the preservation effect will be enhanced with the increase of concentration. However, if the concentration exceeds 2.0%, it will produce drug damage spots and increase the cost. After comprehensive evaluation, 1.0% concentration and 10 minutes soaking time were determined as the best scheme. Under these conditions, the retention rate of vitamin C in spinach was the highest (82.3%), and the antibacterial effect was stable. Dynamic soaking (assisted by ultrasonic) can shorten the treatment time to 7 minutes, significantly improve the efficiency, and is suitable for industrial production.

5.3 Verification and Stability Study of Fresh-keeping Effect

5.3.1 Repetitive Tests

In order to see if our optimization method is reliable, we did three groups of repeated experiments, each with 30 samples, and strictly controlled the temperature (4±1℃) and humidity (90%±5%). The experimental results showed that the weight loss rate, yellowing rate and the coefficient of variation of total bacteria of spinach did not exceed 5% under the best formula and technological treatment. Compared with untreated spinach, the sensory score of the optimized group has been stable at 8.5 points, and the degradation speed of nutrients (such as chlorophyll) has also obviously slowed down ($P<0.01$). These data tell us that the optimized fresh-keeping formula and technology are very stable and practical, and can provide technical help for large-scale production.

5.3.2 Long-term Preservation Stability Test

In this part, we do this test to simulate the real environment and see the effect of the improved preservative during the 30-day storage period. The data show that the spinach in the experimental group still has commercial value after 30 days, its weight loss rate is less than 8%, and the total bacterial count is less than 4 log cfu/g. In contrast, the vegetables in the control group showed serious deterioration on the 15th day. We also did an accelerated experiment (temperature 25℃, relative humidity 60%), and the results showed that the retention rate of effective components of the preservative exceeded 85%, and there was no delamination or precipitation. In GC-MS analysis, we did not detect harmful volatile substances, which indicated that this compound preservative had no safety risk in long-term storage. The research shows that the optimized preservative system can effectively preserve green leafy vegetables and meet the fresh-keeping needs from production to consumption.

6. Conclusion

In this study, we systematically studied the application of compound natural preservative in the preservation of green leafy vegetables, hoping to provide a safe and efficient scheme for the preservation of green leafy vegetables. The main findings can be summarized as follows.

In the process of preparing and identifying the compound natural preservative, we screened and analyzed many natural raw materials, and finally determined the best combination of plant extracts and microbial metabolites, which is safe and effective against bacteria. The results of composition analysis, structure identification and performance test show that the retention rate of effective components is high and the stability is good. This laid a solid foundation for the following fresh-keeping application.

In terms of practical effect, the conventional fresh-keeping test of green leafy vegetables shows that this compound natural preservative can effectively delay the decline of vegetable appearance and flavor quality, reduce the loss of beneficial components such as vitamin C and soluble sugar, and show great inhibitory effect on pathogenic microorganisms such as Escherichia coli and mold.

In the aspect of strategy optimization, the ideal proportion of each component is determined by orthogonal experiments. We also used low-temperature storage technology to improve the process, which greatly improved the preservation effect. This improved method shows strong universality and can provide technical support for large-scale preservation of green vegetables.

The application value of compound natural preservatives is very prominent, because their safe and effective characteristics just conform to the trend of modern food preservation, showing great promotion potential. The results of this study provide an important theoretical basis for the development and practical application of natural preservatives.

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