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EFFECTS OF 'LAOYABAN' FLAVONOIDS ON FRESHNESS OF 'HONGYAN' STRAWBERRY

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Abstract

Flavonoids were prepared from the leaves of 'LAOYABAN'. The ability of 'Hongyan' strawberry soaked with different flavonoids concentrations of 'LAOYABAN' at 5, 10 and 20 mg/mL for 5 min to affect freshness was determined. Simultaneously, the antimicrobial activity was tested to explore its ability to maintain freshness. The results showed that the flavonoids of 'LAOYABAN' reduced the weight loss rate and decay rate of 'Hongyan' strawberries after harvesting. There was also a reduction in the loss of soluble sugar and vitamin C (**Vc**) content. In addition, the flavonoids maintained the activity of SOD, reduced the formation of MDA substance, and had a certain antiseptic and fresh maintenance effect on 'Hongyan' strawberry following harvest. When the concentration of flavonoids was 100 mg/mL, the diameter of the inhibitory zone for *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis* and strawberry mixed bacteria (isolated from rotten 'Hongyan' strawberries) was 12.4 mm, 11.2 mm, 10.8 mm and 8.3 mm, respectively. The minimum inhibitory concentration (MIC) of flavonoids from 'LAOYABAN' against each of these four bacteria was 6.25, 12.5, 25 and 50 mg/mL, respectively. Therefore, the fresh-keeping effect of 'LAOYABAN' flavonoids on 'Hongyan' strawberry is achieved through their bacteriostatic activity and anti-oxidant activity.

Key words: LAOYABAN, Flavonoids, Bacteriostasis, Strawberry preservation.

Introduction

The demand for fresher fruit and vegetable products is increasing, (Khanum *et al*., 2007, Chauncey *et al*., 2019). Most fruits and vegetables are fresh agricultural products, so the industry is actively seeking new preservation technology to improve the freshness period in order to enhance the value and competitiveness on the market (Huang *et al*., 2002; Zhang *et al*., 2012). At present, physical preservation and synthetic chemical preservatives are mainly used to preserve fruits and vegetables (Eklund, 2010; Zhang *et al*., 2012; Yang *et al*., 2013). However, physical preservation involves high energy consumption and high costs, while chemical preservatives are mostly synthesized artificially, which results in some toxicity and side effects (Rai *et al*., 2016). As a result, there is a focus on discovering safe and reliable natural preservatives. Extracting non-toxic, non-polluting and non-side effects natural preservatives from natural plants has become an inevitable trend of fruit and vegetable preservation (Galal, 2006; Hugo & Hugo, 2015; Tak *et al*., 2005).

Studies have shown that flavonoids can be used as natural preservatives with good bacteriostatic effect, which provides a broad space for its development and application in the field of preservation, especially for fruits and vegetables (Ma *et al*., 2011; Pérezgregorio *et al*., 2011; Jia *et al*., 2018). Flavonoids are natural organic compounds and more than 8,000 kinds have been discovered (Sasaki *et al*., 2014). They generally exist in plants in the form of chemical complexes (Wang *et al*., 2013). The health benefits of flavonoids are so significant that their importance cannot be overemphasized, and include anti-oxidation, anti-inflammation, anti-allergy properties and bacteriostasis (Pandey *et al*., 2016; Xiao *et al*., 2019).

'LAOYABAN' (*Tulipa edulis*) is a perennial herb belonging to the genus Tulipa of Liliaceae (Ma *et al*., 2014). The bulb of 'LAOYABAN' (*Pleurotus edulis)* is a Chinese medicinal compound that has the functions of bacteriostasis, detoxification and resolving blood stasis (Ma *et al*., 2014). It mainly treats sore throat, carbuncle, sore and postpartum stasis. In recent years, 'LAOYABAN' have been found to be rich in colchicine and other alkaloids, which are effective anti-cancer substances and have significant therapeutic effects in the treatment of breast diseases (Lee *et al*., 2010).

At present, the research of 'LAOYABAN' mainly focuses on biological characteristics, cultivation and breeding. However, there has been minimal study of flavonoids and their functions (Bing & Zhang, 2008; Yang *et al*., 2015; Sun *et al*., 2016). Therefore, this paper studies the application of 'LAOYABAN' flavonoids in strawberry preservation and discusses the mechanism of its preservation through the determination of bacteriostatic activity, in order to provide a reference for maintaining the freshness of strawberries and other fruits and vegetables. This paper also provides a theoretical basis for the application of 'LAOYABAN'flavonoids for fruit and vegetable preservation.

Materials and Methods

Escherichia coli, Bacillus thuringiensis, Staphylococcus aureus, and 'Hongyan' strawberries (collected from Fangqiuhu Farm, Fengyang County, Anhui Province, China), strawberry mixed bacteria (isolated from the surface of rotten "Hongyan" strawberries) were used.

Preparation of flavonoids from 'LAOYABAN': A total of 100g 'LAOYABAN' powder was weighed and extracted according to the extraction process (65% ethanol, ratio of material to liquid 1:20 (g/mL), temperature 65˚C, extraction time 80 min). The extract was centrifuged for 15 minutes at 4,500 rpm, precipitated, and extracted again. It was then combined with supernatant concentrated by rotary evaporation to obtain the flavonoid concentrate of 'LAOYABAN'. D-101 macroporous resin pretreated with absolute ethanol was packed into a column (2.6*50 cm) and three times as much 65% ethanol was added. The flavonoid concentrate was sampled and eluted by 65% ethanol. The flavonoid-containing components were collected then combined with the eluent, concentrated by rotary evaporation, and freeze dried.

Grouping and treatment: A small amount of 'Hongyan' strawberry was randomly extracted so the initial physiological and biochemical indicator values could be determined 3 times in parallel. The experiment was divided into groups: blank control; distilled water control; treatment group 1, treatment group 2 and treatment group 3. Treatment groups 1, 2 and 3 corresponded to 5, 10 and 20 mg/mL of flavonoids from 'LAOYABAN'. The blank control group of strawberries did not undergo any treatment, while the other 4 groups were soaked with strawberries in distilled water and 5, 10, 20 mg/mL 'LAOYABAN' flavone solution for 5 minutes. This mixture was then dried and stored at room temperature to determine shelf life. The related indexes were determined per day.

Determination of physiological and biochemical indexes of strawberry: The weightlessness rate of strawberry was determined by a weighing method. Three strawberry weightlessness rates were selected in each group and the average value was taken. The weightlessness rate was repeated three times in parallel.

$$\text{Weightlessness rate} = \frac{A0-Ax}{A0} * 100\ \%$$

where **A0** is the initial quality of strawberries and **Ax** is the quality of each measurement of strawberries.

Determination of corrosion rate: According to the rotten area of strawberry, the rotting rate of strawberries can be divided into four grades: 0 grade without rot; 1 grade with rotten area less than 10% of fruit area; 2 grade with rotten area of 10%~40% and 3 grade with rotten area greater than 40% of fruit area.

$$\text{Deterioration rate} = \frac{\text{Deterioration grade x Number of rotten fruits}}{\text{Number of total fruits}} \text{ x } 100\%$$

Determination of anti-oxidant indicators: The content of Vc in 'Hongyan' strawberry was determined by ultraviolet spectrophotometry while its soluble sugar level was determined by a sulfuric acid-anthrone method. The activity of superoxide dismutase (SOD) in strawberry fruit was determined by a NBT photoreduction method. Malondialdehyde (MDA) was determined by a Thiobarbituric Acid (TBA) method in red strawberries.

Antibacterial Activity test of flavonoids from 'LAOYABAN': Filter paper was punched into a 6 mm diameter with a perforator, and then dried after sterilization by high pressure, humidity and heat. Following this process, the filter paper was dipped in 10% (100 mg/mL) of the flavonoid solution from'LAOYABAN'for one hour with sterile tweezers, and then the leached filter paper was dried. The test bacterial solution was inoculated on the culture medium by smearing, and then the filter paper with the flavonoid solution of'LAOYABAN'was clamped with sterile tweezers and placed in the culture dish. It was incubated at 25˚C for 24 hours then the diameter of the bacteriostasis circle was measured.

Determination of minimum inhibitive concentration of flavonoids from 'LAOYABAN': Using a double dilution method, 100 mg/mL flavone aqueous solution of 'LAOYABAN' was diluted into seven concentration gradients of 100-1.5625 mg/mL with sterile water. Different concentrations of 5mL dilution solution was added to the sterile culture dish and mixed with the culture medium to make the plate even. After cooling, 0.1 mL suspension of the tested bacteria was evenly coated on the plate and placed on the 25 mL plate. The minimum inhibitory concentration of the extract was determined by the corresponding concentration of agaric plate following incubation for 24 hours.

Results

Effects of flavonoids from 'LAOYABAN' on weightlessness rate of 'Hongyan' strawberry: The weight loss rate of 5 different treatments of 'Hongyan' strawberry increased with each successive day, showing that the possibility of water loss after harvesting of 'Hongyan' strawberry is extremely positive, which affects the quality and sales value of these strawberries (Fig. 1). However, the weight loss rate of treatment group 1, 2 and 3 was lower than that of the blank control group and distilled water control group, which indicated that the flavonoids of'LAOYABAN'could slow down the weight loss rate of 'Hongyan' strawberry after harvest.

Effect of flavonoids from 'LAOYABAN' on deterioration rate of 'Hongyan' strawberry: The decay rate of 'Hongyan' strawberry in 5 different treatments increased every day. It can also be seen that the decay rate of 'Hongyan' strawberry increased as time went by after harvesting, which then affected their value. However, the decay rate of 'Hongyan' strawberries in treatment 1, 2 and 3 was significantly lower than that in the blank control group and distilled water control group, indicating that the flavonoids of'LAOYABAN'could reduce the decay of 'Hongyan' strawberries after harvest (Fig. 2).

Effect of flavonoids from 'LAOYABAN' on Vc content of 'Hongyan' strawberry: The Vc content of 'Hongyan' strawberry in 5 different treatments decreased each day. It can also be seen that the Vc content of 'Hongyan' strawberry decreased with the passage of time after harvesting. However, the final Vc content of 'Hongyan' strawberry in treatment 1, 2 and 3 was significantly higher than that of the blank control group and distilled water control group. Thus, flavonoids from 'LAOYABAN' can reduce the loss of Vc content in 'Hongyan' strawberries after harvest (Fig. 3).

Effects of flavonoids from 'LAOYABAN' on soluble sugar content of 'Hongyan' strawberry: The soluble sugar content of 'Hongyan' strawberry in 5 different treatments decreases with each successive day. It is also evident that the soluble sugar content of 'Hongyan' strawberry decreases with the passage of time after harvesting, thus affecting their quality. However, the final soluble sugar content of 'Hongyan' strawberry in treatment 1, 2 and 3 was significantly higher than that of the blank control group and distilled water control group, which indicated that the flavonoids of 'LAOYABAN' could reduce the loss of soluble sugar content of 'Hongyan' strawberries (Fig. 4).

Effects of flavonoids from 'LAOYABAN' on SOD activity of 'Hongyan' strawberry: The SOD activity of 'Hongyan' strawberry in 5 different treatments decreased with the passage of time during testing and also after harvesting. However, the SOD activity of 'Hongyan' strawberry in treatment 1, 2 and 3 was significantly higher than that of the blank control group and distilled water control group, indicating that the flavonoids of 'LAOYABAN' could slow down the loss of SOD activity of 'Hongyan' strawberries (Fig. 5).

Effect of flavonoids from 'LAOYABAN' on MDA content of 'Hongyan' strawberry: The MDA content of 'Hongyan' strawberry in 5 different treatments increased with an increase in days. However, the final MDA content of 'Hongyan' strawberry in treatment 1, 2 and 3 was significantly lower than that of the blank control group and distilled water control group. This shows that the flavonoids of 'LAOYABAN' can retain the cells' normal physiological metabolism for a longer time, which delays the ageing rate of 'Hongyan' strawberries and achieves a certain freshness (Fig. 6).

Inhibitory effect of flavonoids from 'LAOYABAN' on experimental bacteria: Table 1 shows that a 100 mg/mL concentration of flavonoids has an obvious bacteriostatic effect on four tested bacteria, and the diameters of bacteriostatic circles for *Escherichia coli*, *Staphylococcus aureu*s, *Bacillus thuringiensis* and strawberry mixed bacteria are 12.4 mm, 11.2 mm, 10.8 mm and 8.3 mm, respectively.

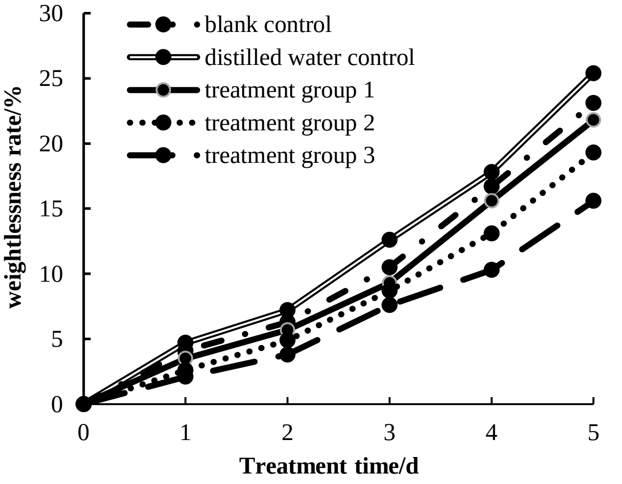

Fig. 1. Effects of Flavonoids from 'LAOYABAN' on Weightlessness Rate of 'Hongyan' Strawberries.

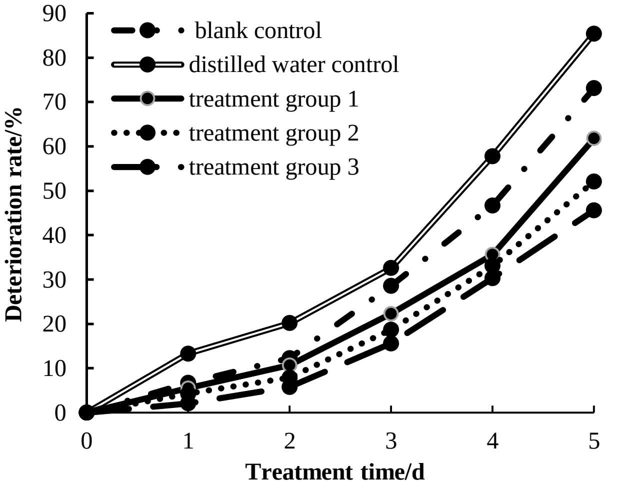

Fig. 2. Effects of Flavonoids from 'LAOYABAN' on the Deterioration Rate of 'Hongyan' Strawberries.

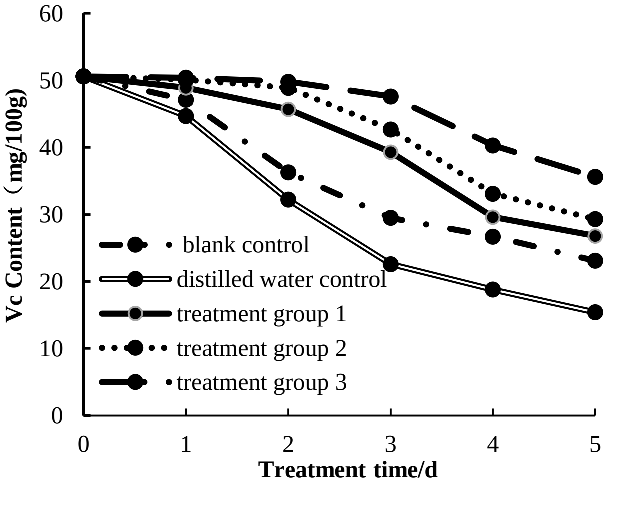

Fig. 3. Effects of Flavonoids from 'LAOYABAN' on Vc Content of 'Hongyan' Strawberries.

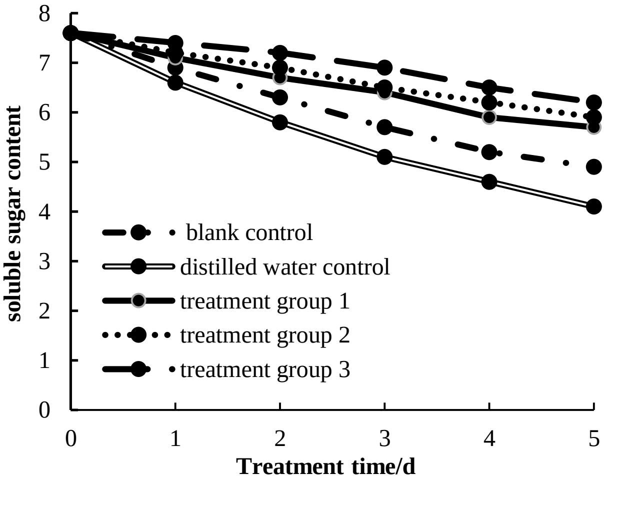

Fig. 4. Effects of flavonoids from 'LAOYABAN' on Soluble Sugar Content of 'Hongyan' Strawberries.

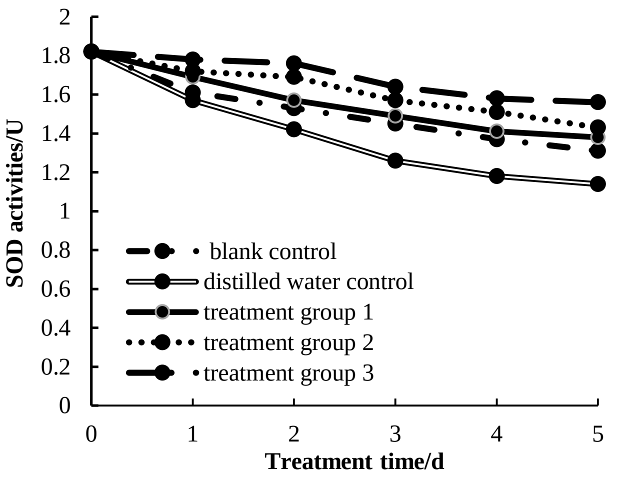

Fig. 5. Effects of flavonoids from 'LAOYABAN' on SOD activity of 'Hongyan' strawberries.

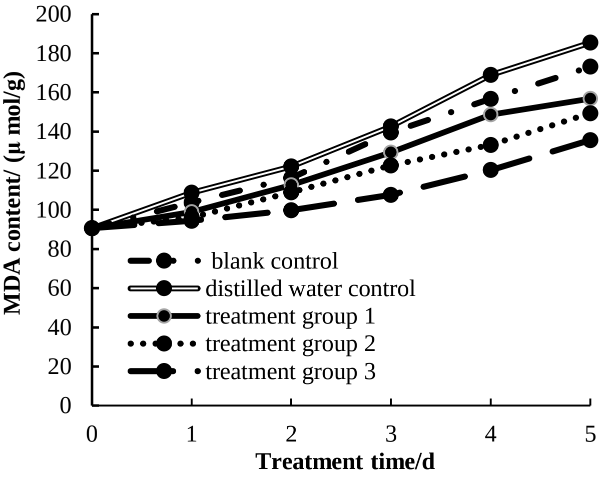

Fig. 6. Effect of flavonoids from 'LAOYABAN' on MDA content of 'Hongyan' strawberry.

Table 1. Inhibitory effect of flavonoids from 'LAOYABAN' on experimental bacteria.

Tested bacteria	Diameter of bacteriostatic circles /mm	Blank control /mm
Escherichia coli	12.4	—
Staphylococcus aureus	11.2	—
Bacillus thuringiensis	10.8	—
Strawberry mixed bacteria	8.3	—

Note: "-" means that there is no bacteriostasis circle

Minimum inhibitory concentration of flavonoids from'LAOYABAN' on tested bacteria: Table 2 shows that the Minimum Inhibitory Concentration (MIC) of aqueous solution of 'LAOYABAN' against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis* and strawberry mixed bacteria was 6.25, 12.5, 25 and 50 mg/ml, respectively. Therefore, the inhibitory effect of flavonoids in 'LAOYABAN' is in the order: *Escherichia coli* > *Staphylococcus aureus* > *Bacillus thuringiensis* > strawberry Mixed Bacteria. This result is consistent with those presented in Table 1. It shows that the flavonoids of 'LAOYABAN' have an inhibitory effect on the tested bacteria.

Table 2. The minimum inhibitory concentration of flavonoids from 'LAOYABAN' on tested bacteria.

Tested bacteria	Concentration of flavonoids / (mg/mL)						
	100	50	25	12.5	6.25	3.125	1.5625
Escherichia coli	—	—	—	—	—	+	++
Staphylococcus aureus	—	—	—	—	+	++	++
Bacillus thuringiensis	—	—	—	+	+	++	++
Strawberry mixed bacteria	—	—	+	+	++	++	++

Note: "-" indicates no mycelial growth
"+" indicates a small amount of mycelial growth
"++" indicates a great amount of mycelial growth

Discussion

With the development of economies, the market demand for strawberries has increased every day, so the preservation of strawberries has become a research focus (Cao *et al*., 2019). The main causes of strawberry decay are the effects of microorganisms and their own respiration (Pesis & Avissar, 2010). Traditional strawberry preservation methods mostly use physical and chemical methods (Kuang *et al*., 2010; Fu *et al*., 2015). However, both methods have certain disadvantages, so there is an urgent need to use safe green biological methods to preserve strawberries. Biological techniques have advantages in the fresh maintaining of fruits and vegetables. It was reported that pomegranate peel extract could reduce the rate of weight loss and rotten fruit of strawberry, and could delay the decline of titratable acid, soluble solids and Vc content of strawberry (Zhang *et al*., 2010). Some researchers demonstrated that the treatments with some Chinese herbal medicine extracts exhibited good performance in decreasing the rate of rot and fruit weight loss, and retarding the decreases of content of total soluble solids, titratable acidity and ascorbic acid. It was also showed that the activities of POD and CAT in peel were significantly improved during later storage (Liu *et al*., 2008).

Flavonoids extracted from plants, which have bacteriostatic activity, can effectively inhibit the bacteria on the surface of postharvest strawberries (Ma *et al*., 2011; Pérezgregorio *et al*., 2011; Jia *et al*., 2018). It was reported that *Curcuma longa* extracts, including flavonoids, alkaloids and quinon, exhibited good antioxidant activity and were found active against different fungal species, including *Mucor mucedo*, *Aspergillus flavus, Aspergillus niger and Saccharomyces boulardii* (Akbar *et al*., 2019). The growth of fungi and other microorganisms can regulate their respiration and thus preserve strawberries. Some scholars used kumquat flavonoids to study the inhibitory effects of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus niger*, *Penicillium Niger* and Mucor. The results showed that except for the growth of *Aspergillus Niger* and *Penicillium Niger*, there was no inhibitory effect on the other four experimental fungi (Lou *et al*., 2016). The results of our study also showed that the flavonoids of 'LAOYABAN' had inhibitory effects on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis* and strawberry mixed bacteria.

A well balanced antioxidant level in plants is very essential in response to environmental stresses (Tariq & Shahbaz, 2020). The study found that the flavonoids of 'LAOYABAN' could significantly (p<0.05) reduce the MDA content and increase the SOD enzyme activity of strawberry, suggesting that the flavonoids could strengthen the antioxidant capacity and delay the senescence process of strawberry fruit.

Flavonoids from 'LAOYABAN' have good antioxidant activity *In vitro* (literature) and certain bacteriostasis effect. These properties lay the foundation for the preservation of flavonoids from 'LAOYABAN. The quality and freshness of strawberries after harvesting are mainly determined by their respiration and microorganisms. Flavonoids from 'LAOYABAN' can weaken respiration and inhibit the growth of microorganisms through bacteriostasis. The chemical structure, toxicological test and cell inhibition of flavonoids from 'LAOYABAN' need to be further studied in order to provide some experimental basis for the production of flavonoids from 'LAOYABAN' as a preservation agent for fruits and vegetables.

Conclusions

Flavonoids from 'LAOYABAN' can reduce the weight loss and decay rate of 'Hongyan' strawberries after harvest. They can also increase sugar content, soluble sugar and Vc content. In fact, they provide better maintenance for the activity of SOD and reduce the formation of MDA substances. Therefore, flavonoids from 'LAOYABAN' have a certain freshness maintenance effect on 'Hongyan' strawberries after harvest and can be used for natural fruit and vegetable preservation. The results showed that 'LAOYABAN' flavonoids had a good antimicrobial effect on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis* and strawberry mixed bacteria, with improved strawberry preservation through bacteriostasis and oxidation resistance.

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