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Isolation and Identification of Bacteria Leading to Microbial Spoilage of Sweet Potato and Screening for Antistaling Agents Running Head

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Abstract: To develop natural preservatives tailored for sweet potatoes, the control effect of essential oils on the spoilage microorganisms of sweet potatoes is uncertain. Therefore, we first isolated and identified the dominant spoilage bacteria from spoiled sweet potatoes. Micrococcus aloeverae, Priestia aryabhattai, and Glutamicibacter arilaitensis were identified through screening, morphological observation, gram staining, and 16S rDNA assays. Subsequently, five plant essential oils (citral, Litseacubeba, parsley, eugenol, and *chinaberry* tree oils) that significantly inhibited the spoilage bacterial species were identified after screening 20 plant essential oils using the zone of inhibition test. Minimum Inhibitory Concentrations (MICs) of *Litsea cubeba*, eugenol, and parsley oils against the above three spoilage bacteria were 320-1280, 640-1280, and 320-640 μL/L, respectively. The three essential oils exhibiting the most favorable inhibitory effects were selected for combinatorial studies. Finally, the response surface methodology was used to optimize the combination concentration of *Litsea cubeba*, eugenol, and parsley oils. The optimum concentration was identified as follows: 320 μL/L for *Litsea cubeba* oil, 716 μL/L for parsley oil, and 1280 μL/L for eugenol oil. At this concentration, the growth of three spoilage bacteria was almost completely inhibited (OD values of 0.067, 0.202, and 0.013). The optimized compound essential oil exhibited potent inhibitory activity against the spoilage bacteria of sweet potatoes. The aforementioned studies may provide important guidance for the development of efficient and broad-spectrum sweet potato preservatives.

Keywords: Sweet Potato Spoilage Bacteria, Isolation and Identification, Plant Essential Oil, Response Surface Methodology

Introduction

Sweet potato (*Ipomoea batatas*) is an annual plant in the family of Convolvulaceae and is an important food crop second only to wheat, rice, maize, and potatoes. Sweet potato is drought-resistant and tolerant of barren soil, enjoys light and warmth, and is highly adaptable, making it suitable for cultivation in areas with sufficient light and heat resources. China is the world's largest sweet potato producer. According to statistics, in 2020, sweet potato production in China was 5126.4×104 t, accounting for 56% of the world's total production (Maeda *et al*., 2022). Due to its sweet taste and richness in starch, carotene, edible fiber, a variety of amino acids, and minerals including calcium, phosphorus, iron, and potassium, sweet potatoes exert anti-cancer, anti-aging, and anti-atherosclerotic effects as well as other health-promoting effects such as enhancing immune function. The sweet potato is known as the "queen of vegetables" and the "longevity vegetable" (Bengtsson *et al*., 2008).

Due to the rich nutritional constituent of sweet potatoes, they are especially vulnerable to infection by spoilage microorganisms, resulting in sweet potato spoilage (Yuan *et al*., 2016), possessing a large loss during storage. Primary disease-causing microorganisms are observed among molds, yeasts and bacteria. Among the microorganisms, the Dominant Spillage Organisms (DSOs) are those that play a dominant role in the deterioration of sweet potato quality during picking,

processing, and storage (Xia *et al*., 2020). Common diseases known to occur during sweet potato storage include bacterial soft rot, black spots, dry rot, and gray mold (Ruengvisesh *et al*., 2020; Nayaka *et al*., 2021).

In recent years, physical or chemical approaches have often been adopted to optimize post-harvest storage of sweet potatoes to minimize losses. Commonly used physical methods include cryopreservation, air conditioning, depressurization, radiation, and heat treatment. The aforementioned methods can delay the onset of disease, temporarily preventing decay and preserving quality (Sharifi and Nayeri Fasaei, 2022). However, such methods are expensive and cannot indefinitely prevent sweet potato infection by pathogenic microorganisms. Physical measures such as low temperatures and chemical agents have also been adopted for preservation (Zhang *et al*., 2021). However, long-term use of chemicals may lead to the development of drug resistance among spoilage bacteria. Moreover, the use of large quantities of chemicals may result in chemical residues on sweet potatoes that are hazardous to human health. With improvements in people's living standards and the “green food” initiative, the discovery of environmentally compatible preservatives with low toxicity and minimal residue has become a research hot spot (Lougraimzi *et al*., 2021). Herein, the development of alternative, safe, and natural preservatives is more urgent. Essential oils are volatile secondary metabolites extracted from plant leaves, seeds, flowers, and roots (most extracts are colorless or light yellow) (Putra, 2021). Plant essential oils have gradually been applied in the food, pharmaceutical, agricultural, cosmetic, and textile industries due to their green nature, safety, broad-spectrum antimicrobial and non-resistance-promoting properties (Nguiam *et al*., 2021). Furthermore, plant essential oils have become the most common biological preservatives, as they are characterized by broad-spectrum antibacterial activities, zero residue, environmental friendliness, and low cost (Jahani *et al*., 2021). Currently, some researchers have focused on the essential oils that control the deterioration of fruits, such as strawberries and peaches (Anonymous, 2021; Zhou *et al*., 2020). However, the control effect of essential oils on the spoilage microorganisms of sweet potatoes is uncertain.

Therefore, in the study, to identify specific essential oils that can control spoilage microorganisms of sweet potato, key spoilage microorganisms were isolated and purified from decaying sweet potatoes and identified by molecular biological characterization. The identified spoilage bacteria were then used as targets to screen for plant essential oils with bacteriostatic or bactericidal activities by zone of inhibition testing and minimal inhibitory concentration. An optimized combination of the three most effective essential oils was developed to obtain the most efficacious bacterial inhibition through response surface methodology. The findings provided a theoretical basis for prophylaxis against infection of sweet potatoes by spoilage bacteria during harvesting and storage and for inhibition of spoilage bacteria on sweet potatoes. They also serve as a theoretical guide for the development of novel sweet potato preservation technologies

Materials and Methods

Strains, Media and Reagents

Three strains of bacteria were isolated from spoiled sweet potatoes by the microbiology laboratory, at the school of biology and food engineering, Huanghuai University.

The essential oil sample library comprised white camphor, thyme, tea tree, wheat germ, citral, *Litsea cubeba*, pumpkin seed, eugenol, *Lavender*, parsley, *Houttuynia cordata*, chrysanthemum, camphor, osmanthus, lemon, *Vetivert*, licorice, *Costus* root, *chinaberry* and myrrh oil (Jiangxi Hengcheng natural fragrance oils Ltd).

Luria-Bertani (LB) broth medium (containing 10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract) was used for strain isolation, purification and storage, as well as liquid seed preparation and screening of anti-bacterial agents (Beijing Aobox biotechnology Co., Ltd.).

Instruments and Equipment

Equipment included a vertical high-pressure steam sterilizer autoclave and electronic balance (Shanghai Sunny Hengping Instrument Co. Ltd.), an optical microscope (Shanghai Wumo Optical Instrument Co.), a constant temperature incubator (Shanghai Bluepard Instruments Co., Ltd.), a constant temperature shaking incubator (Shanghai Zhicheng analytical instrument manufacturing Co., Ltd.) and a sterile workbench (Suzhou Jinghua equipment limited company).

Isolation and Purification of Spoilage Bacteria

Several spoiled sweet potatoes were selected and a total of 10 g of pathological tissues were placed in a triangular vial with 90 mL of sterile saline and glass beads, which was vigorously shaken for 30 min to obtain a sample solution containing spoilage bacteria.

A series of 10-fold dilutions of the sample bacterial solution was prepared and applied to the dilution plate method. Dilutions spanning 10^{-4}-10^{-7} were selected for plating and each group was plated in triplicate. Plates were allowed to stand for 15 min, then inverted and placed in a 37°C incubator for 48±2 h. A streak plate method was adopted for strain purification.

Suspected single colonies were picked and streaked on LB solid medium multiple times until pure strains were obtained. Pure strains were then numbered and stored in a refrigerator at 4°C for later use.

Morphological Observations of Spoilage Bacteria

Isolated and purified spoilage bacteria were plated on LB agar plates and incubated at 37°C for 24 h. Single colonies were selected to observe color, shape, transparency, smoothness, wetness and neatness of edges. Then gram staining was performed and cell morphology was observed through a microscope.

Molecular Biological Characterization of Spoilage Bacteria

The three purified strains of sweet potato spoilage bacteria were sent to Wuhan ServiceBio Biotechnology Co. Ltd. for 16S recombinant Deoxyribonucleic Acid (rDNA) sequencing. Sequence similarity was searched in standard databases of the National Center for Biotechnology Information (NCBI) BLAST using highly similar sequences.

Assessment of the Antibacterial Activity of 20 Plant Essential Oils

To evaluate whether plant essential oils have antibacterial activities, the antibacterial activities of 20 plant essential oils against the three isolated strains of sweet potato spoilage bacteria were determined by zone of inhibition testing to identify essential oils with high antibacterial activity (Proto *et al*., 2022). The 20 plant essential oils were first emulsified with 1% tween-80, each to a final concentration of 5%. The essential oil emulsions were ultrasonicated for 5 min to achieve thorough emulsification. Activated spoilage bacteria were then uniformly spread onto LB solid medium. After standing for 15 min to allow bacterial media to be fully absorbed, four small equally spaced holes were made with a sterile yellow pipet tip (d = 5 mm), and the medium in the holes was removed to form wells into which essential oils could be placed. 50 μL of plant essential oil emulsion was added to the experimental group and an equal amount of 1% aqueous solution of tween-80 was added to the control group. Finally, the diameters of the inhibition circles were determined using the criss-cross method.

Determination of Minimal Inhibitory Concentration (MIC)

To further determine the antibacterial activity of the above plant essential oils, activated target bacterial solutions were diluted with saline. A turbidimetric tube with 0.5 McFarland turbidity (10^8 cfu/mL) was used for turbidimetry. Samples were then diluted 10-fold in LB broth and set aside for later use (Valková *et al*., 2021).

Plant essential oil samples were prepared by two-fold dilution: 13 test tubes were sequentially numbered and a two-fold dilution method was adopted. Five mL of essential oil at a concentration of 20.48 μL/mL and 5 mL of broth were added to the first tube and mixed well. Five mL of each first sample was drawn from the first tube to the second tube, which already contained 5 mL of broth, followed by thorough mixing. Serial two-fold dilutions were performed on tube 11. Finally, 5 mL of sample solution was drawn from tube 11 and discarded. Therefore, the respective concentrations of the first 11 tubes in descending order were 10.24, 5.12, 1.28, 0.64, 0.32, 0.16, 0.08, 0.04, 0.02 μL/mL and 0.01 μL/mL. Then 50 μL of the above-mentioned bacterial suspension was added to each of tubes 1-12, with tube 12 serving as a positive control and tube 13 serving as a negative control. The tubes were placed in an incubator at 37°C for 24 h. The lowest concentration in which the drug completely inhibited the growth of bacteria in the test tube was considered the MIC (El Khetabi *et al*., 2022).

Optimization of a Combination of the Three Most Potent Essential Oils Using Response Surface Methodology

To obtain the optimal combination concentration of essential oils based on the result of MIC, using the above zone of inhibition method to determine MIC values, three plant essential oils with high antibacterial activity were identified. Design-expert 8.06 was used to design three factors and three levels and the optical density at 600 nm (OD_{600}) of the three spoilage bacteria was used as the response value for the analysis to determine the optimal concentration of each in a combination of the three plant essential oils. The test design is presented in Table 1. Design-expert offers several designs depending on the factors (*Litsea cubeba* oil, parsley oil, Eugenol oil) and the objective is to find a minimum in the design space. These designs are built algorithmically to provide the most accurate estimates of the model coefficients.

Data Analyses

Three biological replicates were performed for all experiments in the study. Design expert was used to design the response surface analysis and graph pad prism was used to plot the graphs.

Table 1: Experimental factors and levels

	Levels				
Factors	1.68	-1	0	1 Factors	1.68
A: *Litsea cubeba* oil (μL/L)	190	320	640	1280	2150
B: Parsley oil (μL/L)	190	320	640	1280	2150
C: Eugenol oil (μL/L)	380	640	1280	2560	4300

Note: "-1" indicates the lower level of the factor; "+1" indicates the upper level; "0" indicates the middle

Results and Discussion

Isolation and Characterization of Sweet Potato Spoilage Bacteria

We followed the procedure described above to obtain specific strains of sweet potato spoilage bacteria. Plates with a total dilution of 10^{-6} were selected based on the number of colonies for the subsequent isolation and purification of bacterial strains. Bacteria isolated from spoiled sweet potatoes were then separated by repeated plate streaking on LB solid medium until pure single colonies were obtained and numbered, as demonstrated in Fig. 1. Colonies of strain XC03 are milky white, large and flat, with sticky and smooth surfaces as well as neat edges.

Moreover, XV04 colonies are golden yellow, small, smooth and slightly transparent with neat edges whereas, XB05 colonies are milky yellow, small and uniform, with moist and elevated surfaces and neat edges.

To observe the cellular morphology of each strain of spoilage bacteria, the above strains were subjected to gram staining, and the results are displayed in Fig. 2. XC03 was ellipsoidal and lacked spores and flagella. XV04 were short rods without capsules. XB05 were short rods with capsules but without flagella. XV04 gram stained red, indicating that it was gram-negative. The other two strains both stained blue-purple, indicating that they were gram-positive.

To definitively identify the above three spoilage bacteria, samples of each were sent to Wuhan ServiceBio Biotechnology Co. Ltd. for 16 S rDNA sequencing. The obtained sequences were submitted to the NCBI database for sequence alignment to obtain homologous sequences. Comparative 16S rDNA gene sequence analysis showed that strains XC03, XV04, and XB05 were most closely related to members of the genus *Micrococcus aloeverae*, *Priestia aryabhattai*, *Glutamicibacter arilaitensis*, respectively. The sequence similarity values of XC03, XV04, and XB05 to recognized members of the above genus were 100, 99.86 and 98.92%, respectively. We thus identified three bacteria that underlie sweet potato spoilage: *Micrococcus aloeverae*, *Priestia aryabhattai*, and *Corynebacterium glutamicum*. Currently, the main spoilage bacteria found in fruits, vegetables, and agricultural products are fungi and yeast (Li *et al*., 2017), such as *Penicillium* and *Sporobolomyces*. However, in this study, spoilage bacteria were isolated and obtained, which enriched the library of the spoilage microorganisms.

Determination of the Inhibitory Activity of 20 Plant Essential Oils Against the Three Identified Spoilage Bacteria Strains

To develop efficient, safe, and stable natural sweet potato preservatives, we used three bacteria strains isolated from spoiling sweet potatoes that contribute to spoilage as targets of a screen for essential oils with potent antibacterial activity. We selected 20 plant essential oils for our library based on a literature review and determined their inhibitory activities against the above spoilage bacteria through the zone of inhibition testing to identify the essential oils with the most potent inhibitory activities. The results are presented in Table 2. Based on the diameter of the inhibitory zones, 10 of the 20 plant essential oils (thyme, citral, *Litsea cubeba*, eugenol, *Lavender*, parsley, *Houttuynia cordata*, linalool, *Osmanthus* and *Chinaberry* oils) demonstrated significant inhibitory effects against all three sweet potato spoilage bacteria.

Among the ten, citral, *Litsea cubeba*, and PARSLEY oils demonstrated the most potent inhibitory effects against *Micrococcus aloeverae*, with respective inhibitory zone diameters of 24.6±0.3 mm, 30.2±0.2 mm, and 24.6±0.2 mm. Parsley and *Chinaberry* tree oils demonstrated the most potent inhibitory effects against *Priestia aryabhattai*, with respective inhibitory zone diameters of 22.3±0.2 mm and 20.0±0.3 mm. *Litsea cubeba*, eugenol, and parsley oils were effective against *Corynebacterium glutamicum*, with respective inhibitory zone diameters of 34.4±0.3 mm, 22.5±0.2 mm, and 22.1±0.3 mm. Five essential oils (citral, *Litsea cubeba*, eugenol, parsley, and *Chinaberry* tree oil) were thus selected for MIC determination.

Determination of Minimum Inhibitory Concentrations of Essential Oils with High Antibacterial Activities

To determine MIC values for the above five plant essential oils with high antibacterial activities, we adopted a two-fold serial dilution method. The results are presented in Table 3. The respective MICs of citral, *Litsea cubeba*, eugenol, parsley, and *Chinaberry* tree oils against *Micrococcus aloeverae* were 320, 320, 640, 320, and 1280 μL/L. The respective MICs of the above five oils against *Priestia aryabhattai* were 1280, 1280, 1280, 1280, 640 and 320 μL/L. The respective MICs against *Glutamicibacter arilaitensis* were 640, 320, 640, 320 and 1280 μL/L. The MICs were consistent with previous articles (Kontaxakis *et al*., 2020).

Fig. 1: Gram status and cell morphology of three strains of spoilage bacteria isolated from spoiled sweet potatoes

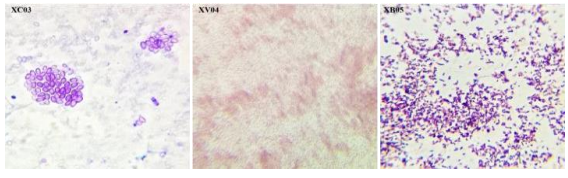


Fig. 2: Microscopic morphology of the three strains of spoilage bacteria isolated from spoiled sweet potatoes

Table 2: Inhibitory zone diameters (mm) of 20 plant essential oils against sweet potato spoilage bacteria

	Spoilage bacteria		
Essential oils	*Micrococcus aloeverae*	*Priestia aryabhattai*	*Glutamicib acter arilaitensis*
White camphor	-	-	-
Thyme	20.3±0.2	10.5±0.3	20.6±0.2
Tea tree	14.4±0.3	-	1.2
Wheat germ	-	-	-
Citral	24.6±0.3	11.0±0.2	18.1±0.2
Litsea cubeba	30.2±0.2	14.3±0.1	34.4±0.3
Pumpkin seed	-	-	-
Eugenol	20.6±0.1	12.4±0.3	22.5±0.2
Lavender	16.4±0.2	8.1±0.1	18.7±0.1
Parsley	24.6±0.2	22.3±0.2	22.1±0.3
Houttuynia Cordata	12.6±0.1	10.1±0.3	11.8±0.3
Chrysanthemum	-	-	-
Camphor	15.9±0.3	14.2±0.2	1.78±0.3
Osmanthus	11.7±0.3	11.8±0.1	12.30±0.3
Lemon	-	-	-
Vetivert	-	-	-
Licorice	-	-	-
Costus root	9.8±0.2	-	-
Chinaberry	14.6±0.2	20.0±0.3	14.2±0.2
Myrrh oil	12.4±0.1	-	-

Note: The unit was "mm". Oxford cup diameter is included in the measurement result; "-" indicates no inhibition circle

Table 3: MIC of five plant essential oils against three strains of sweet potato spoilage bacteria

		Content (μL/L)						
Spoilage bacteria	Essential oils	5120	2560	1280	640	320	160	80
	Citral	+	+	+	+	+	-	-
Micrococcus aloeverae	*Litsea cubeba*	+	+	+	+	+	-	-
	Eugenol	+	+	+	+	-	-	-
	parsley	+	+	+	+	+	-	-
	Chinaberry	+	+	+	-	-	-	-
	Citral	+	+	+	-	-	-	-
	Litsea cubeba	+	+	+	-	-	-	-
Priestia aryabhattai	Eugenol	+	+	+	-	-	-	-
	parsley	+	+	+	+	+	-	-
	Chinaberry	+	+	+	+	-	-	-
	Citral	+	+	+	+	-	-	-
	Litsea cubeba	+	+	+	+	+	-	-
Glutamicibacter arilaitensis	Eugenol	+	+	+	+	-	-	-
	parsley	+	+	+	+	+	-	-
	Chinaberry	+	+	+	-	-	-	-

Note: "+" indicates a significant antibacterial effect; "-" indicates no antibacterial effect

The above results indicated that different plant essential oils have different antibacterial effects on the same bacteria, while each essential oil demonstrated different antibacterial activities on different microorganisms. Plant essential oils are mainly composed of terpenes, alcohols, aldehydes, ketones, acids, and esters and vary greatly in composition. The complexity and variability of the composition of plant essential oils confer selective antimicrobial activities (Proto *et al*., 2022).

Optimization of a Combinatorial Essential Oil Formulation Using Response Surface Methodology

To obtain an efficient selective composite natural preservative for sweet potatoes, three essential oils (*Litsea cubeba* oil, eugenol oil, and carvacrol), which demonstrated favorable antibacterial effects, were selected based on the above findings for combinatorial studies. Response surface analysis was designed using design expert software. The OD_{600} values of the three

strains of spoilage bacteria were used as the response values. The results are displayed in Table 4. Analysis of Variance (ANOVA) was performed on the results. ANOVA results are presented in Table 5.

In the above models, a p-value <0.05 indicated that the response surface model was significant. The coefficients of determination R^2 were 0.9990, 0.9016 and 0.9025, respectively, indicating that the regression models were well-fitted and that the models may be used to analyze and predict the response values. The response surface analysis plot was made based on the above results, as presented in Table 4.

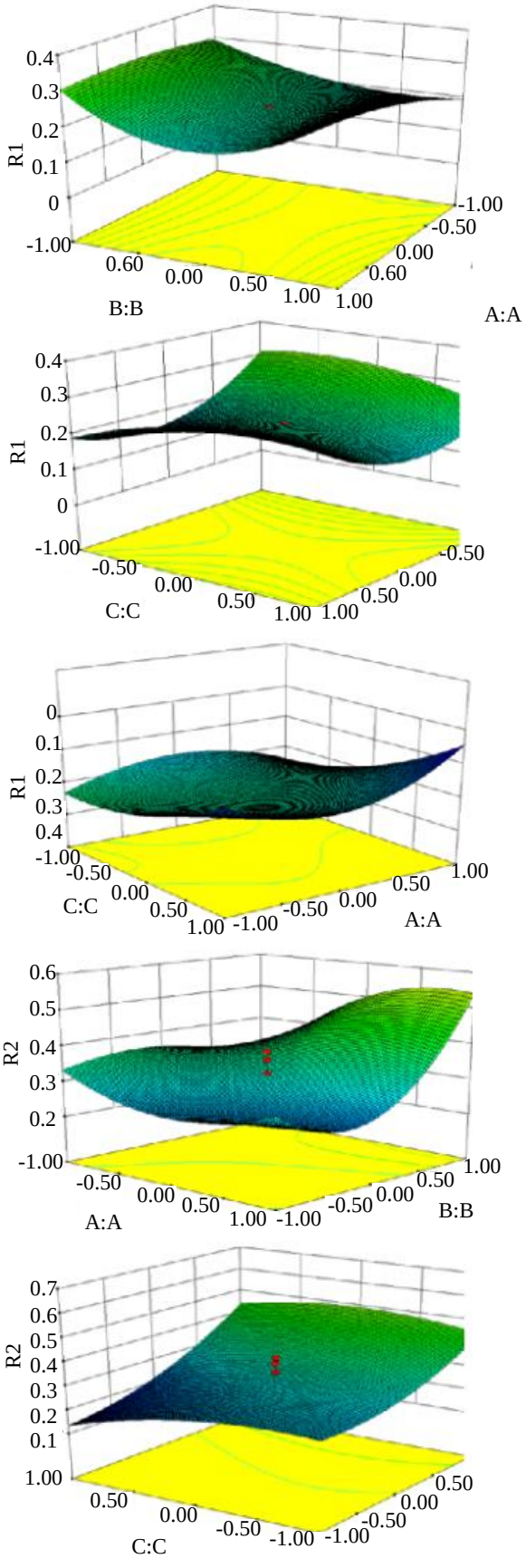
Figure 3. The opening of the parabola fitted to the OD_{600} of the three strains of spoilage bacteria as the response values faced upwards, indicating the presence of a minimum value and that the response surface covered the area where the minimum OD of the spoilage bacteria was located. Based on this model, the optimal essential oil concentrations for combinatorial use were 320 μL/L for *Litsea cubeba* oil, 716 μL/L for parsley oil, and 1,280 μL/L for eugenol oil. The predicted OD_{600} values under such conditions were 0.078 for R_1, 0.242 for R_2 and 0.008 for R_3. The optimum concentration of *Litsea cubeba* oil, parsley oil, and eugenol oil is higher than that of previously published articles. However, the optimum concentration in this study could simultaneously inhibit three spoilage bacteria (*Micrococcus aloeverae*, *Priestia aryabhattai*, and *Glutamicibacter arilaitensis*), previous articles usually targeted a certain spoilage microorganism (Li *et al*., 2017).

Table 4: Design and results of the response surface analysis

No.	A	B	C	OD_{600}	OD600	OD_{600}
1	1.68	0.00	0.00	0.142	0.229	0.074
2	0.00	0.00	0.00	0.213	0.216	0.065
3	0.00	0.00	0.00	0.213	0.247	0.065
4	1.00	-1.00	-1.00	0.450	0.251	0.065
5	0.00	0.00	1.68	0.091	0.149	0.064
6	0.00	0.00	0.00	0.213	0.321	0.067
7	0.00	0.00	0.00	0.213	0.382	0.069
8	-1.00	1.00	1.00	0.081	0.168	0.065
9	0.00	0.00	0.00	0.213	0.358	0.065
10	0.00	-1.68	0.00	0.482	0.310	0.184
11	-1.00	1.00	-1.00	0.231	0.213	0.119
12	-1.00	-1.00	1.00	0.200	0.213	0.150
13	0.00	1.68	0.00	0.414	0.718	0.061
14	1.00	-1.00	1.00	0.079	0.221	0.151
15	-1.68	0.00	0.00	0.181	0.405	0.006
16	1.00	1.00	1.00	0.282	0.558	0.072
17	-1.00	-1.00	-1.00	0.312	0.304	0.281
18	0.00	0.00	0.00	0.213	0.357	0.065
19	1.00	1.00	-1.00	0.295	0.377	0.065
20	0.00	0.00	-1.68	0.078	0.270	0.058

Table 5: Response surface analysis ANOVA results

Response	p-value	R-squared
R1	<0.0001	0.9990
R2	0.0434	0.9016
R3	0.0423	0.9025



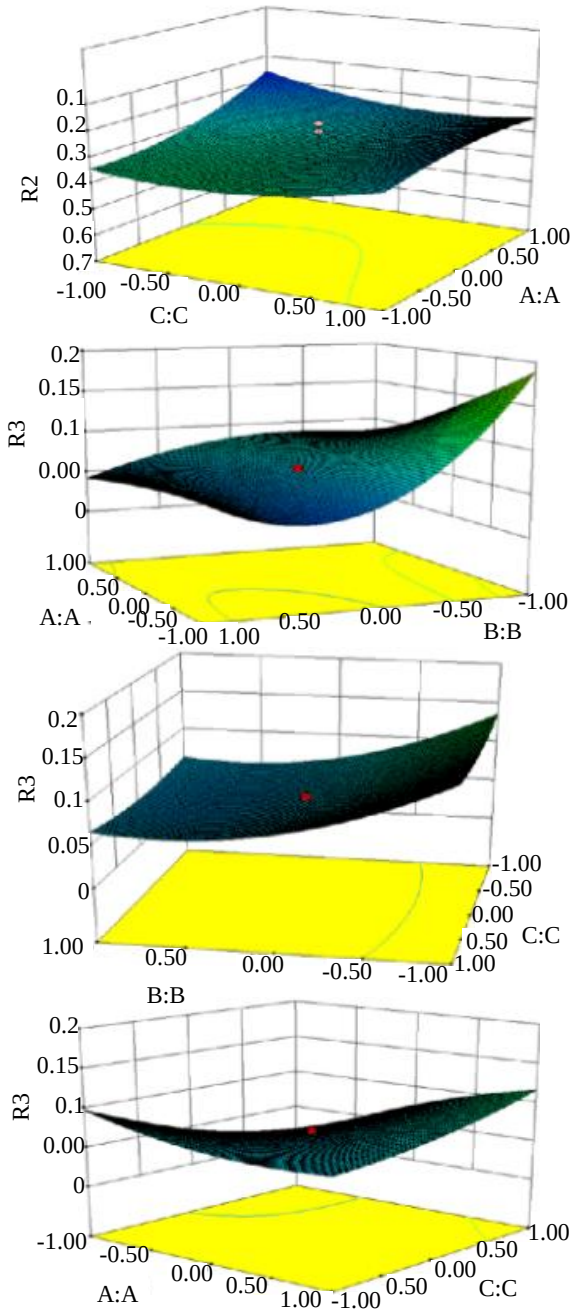


Fig. 3: Response surface analysis of interactions between essential oils; Note: R1, R2, and R3 represent the respective OD_{600} values for *Micrococcus aloeverae*, *Priestia aryabhattai*, and *Glutamicibacter arilaitensis*. A, B, and C represent *Litsea cubeba*, parsley, and eugenol oils, respectively

Table 6: Verification of the antibacterial effect of the compound essential oils optimized by response surface analysis

Spoilage bacteria	OD_{600}	cfu/mL
Echinococcus circumscriptus	0.067	2
Bacillus polypanicus	0.202	7
Corynebacterium glutamicum	0.013	0

To verify the antibacterial efficacy of the compound essential oil optimized through response surface analysis, essential oils combined at the above concentrations (320 μL/L of *Litsea cubeba* oil, 716 μL/L of parsley oil and 1,280 μL/L of eugenol oil) were added into LB liquid medium. The medium was then inoculated with the three spoilage bacteria separately to test the antibacterial effect of the compound oil against each sweet potato spoilage bacteria using the incubation method for liquid seeds. Table 6, the growth of the three spoilage bacteria in the triangular vials was completely inhibited, with respective OD values of 0.067, 0.202 and 0.013, consistent with the values predicted by response surface analysis. Another 100 μL of the above culture solution was spread onto LB solid medium to measure the viable bacteria count and the results suggested nearly no growth. Therefore, the compound essential oil had high inhibitory activity against the sweet potato spoilage bacteria, providing important guidance for the subsequent development of efficient, broad-spectrum sweet potato preservatives.

Conclusion

In the study, spoiled sweet potatoes were used as a raw material to isolate and purify key spoilage bacteria through dilution plating and streak plate isolation. Three strains of spoilage bacteria were isolated. Microscopic analysis revealed that one strain was gram-negative while the remaining two strains were gram-positive. Molecular identification indicated that the spoilage bacteria were *Micrococcus aloeverae*, *Priestia aryabhattai* and *Glutamicibacter arilaitensis*. Then, preliminary screening of 20 plant essential oils was performed through zone inhibition testing to identify essential oils that could inhibit sweet potato spoilage bacteria. Five effective essential oils (citral, *Litsea cubeba*, parsley, eugenol and *Chinaberry* oils) were identified. Moreover, MIC values were determined using a two-fold serial dilution method. Three essential oils (*Litsea cubeba* oil, eugenol oil and parsley oil) displayed the most potent inhibitory effects and were selected for combinatorial testing. Finally, the MIC measurements of the three plant essential oils were used as the center point and the respective optimal compound concentrations of the three essential oils according to response surface analysis were 320 μL/L for *Litsea cubeba* oil, 716 μL/L for parsley oil and 1280 μL/L for eugenol oil. The optimum concentration in this study could simultaneously inhibit three spoilage bacteria (*Micrococcus aloeverae*, *Priestia aryabhattai* and *Glutamicibacter arilaitensis*), however, previous articles usually targeted a certain spoilage microorganism. The aforementioned studies may provide important guidance for the development of

efficient and broad-spectrum sweet potato preservatives. the beginning of a sentence.

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Author's Contributions

Daoqi Liu, Mingcheng Wang and Enzhong Li: Designed the whole and wrote the final manuscript.

Huili Liu and Yuanyuan Dang: Carried out all experiment, data collection and manuscript edited.

Huili Xia and Shuyu Shi: participated in data analysis.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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