JOURNAL OF
EMERGING INVESTIGATORS

Article

The effect of common food preservatives on the growth of bacteria

Gan Yulin[1], Samuel Gan[1]
[1] Guangzhou Foreign Language School, Guangzhou, Guangdong, China

SUMMARY

Food preservation is a common topic in everyday life. However, the individual effects of different preservatives and the combined effects of multiple preservatives present excellent subjects for research because the combined effects of preservatives have not been studied in depth. The main purpose of this study was to determine the optimal solution for preservatives by combining data modeling with biochemical experiments. We hypothesized that sodium benzoate, sodium nitrite, and vitamin E would inhibit *Staphylococcus aureus* and *Salmonella enterica* growth, and that sodium benzoate, sodium nitrate, and sodium bisulfite would inhibit *Bacillus subtilis* growth. Different preservatives were selected for the different bacterial species based on the preservatives' mechanisms of inhibition. Through systematic testing of different concentrations, we identified the optimal amount of each preservative needed to effectively inhibit bacterial growth while ensuring compliance with current food safety regulations. We found that single preservatives may be inadequate for inhibiting bacterial growth, with vitamin E showing poor effectiveness. Sodium benzoate and sodium nitrite exhibited good antibacterial effects against *Staphylococcus aureus*, while sodium benzoate had the best inhibitory effect on *Salmonella enterica*, followed by sodium nitrite. Sodium bisulfite showed the best inhibitory effect on *Bacillus subtilis*, followed by sodium benzoate. The study provides a pathway for future exploration of preservative compounding to optimize their effectiveness against bacterial growth and prevent food spoilage.

INTRODUCTION

Food spoilage is a significant challenge facing the global food industry, causing substantial economic losses and potentially endangering consumer health. Bacteria such as *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis* are the primary microorganisms responsible for food deterioration. Preservatives are widely used to extend shelf life and ensure product safety. However, the inhibitory effects of different preservatives on various bacteria may vary, making it crucial to thoroughly investigate their antibacterial mechanisms and efficacy (1, 2).

Food spoilage is primarily caused by bacterial growth, with chemical preservatives being used as an inhibitor despite potential adverse effects on human health (3). Long-term excessive intake of chemical preservatives may have a negative impact on liver and kidney function, cause gastrointestinal discomfort, such as abdominal pain and diarrhea, and may also interfere with the normal function of the human endocrine system (3). The concentration of general preservatives ranges from 0.05wt% to 2wt%, depending on local regulations and different foods (4). These concentrations are based on government regulations to limit the dosage of preservatives **(Table 1)**. Thus, it is crucial to research effective bacterial inhibition using preservatives at lower concentrations. Comparing the effects of different preservatives on the same bacterial strain and examining how a single preservative inhibits different bacteria can yield valuable insights into potential mechanisms of action (5).

In this study, we hypothesized that sodium benzoate, sodium nitrite, and vitamin E would inhibit *Staphylococcus aureus* and *Salmonella enterica* growth, and that sodium benzoate, sodium nitrate, and sodium bisulfite would inhibit *Bacillus subtilis* growth. We employed two standard methods to evaluate antimicrobial efficacy. The paper diffusion method measured inhibition zones by placing preservative-saturated paper disks on bacterial lawns, where larger zones indicated stronger antimicrobial activity. We then used the minimum inhibitory concentration (MIC) assay, which involved exposing bacteria to decreasing preservative concentrations to determine the lowest concentration that prevented visible bacterial growth. Linear regression and optimization models were then used to calculate the optimal ratio of multiple preservatives in accordance with safety standards. These models utilized experimental data and other literature data, aiming to reduce preservative use, cut costs, minimize health risks, and decrease chemical waste production. In this study, different preservatives were selected for each bacteria based on the commonly used preservatives in the production of foods where these bacterial species are likely to occur in industrial production. The optimization obtained was based on the optimal mixture of these five preservatives. Our optimization model recommended a preservative composition of 73.3211 μg/mL sodium benzoate, 11.0424 μg/mL sodium nitrite, 218.958 μg/mL vitamin E, and 12.0742 μg/mL sodium bisulfite, which should effectively inhibited bacterial growth while meeting food safety standards. Based on predictive analysis from the optimization model, this mixture is anticipated to demonstrate significant antimicrobial activity against *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis*, while utilizing reduced preservative concentrations.

RESULTS

First, we investigated the effect of each preservative independently. We used sodium benzoate, sodium nitrite, and vitamin E to inhibit *Staphylococcus aureus* and *Salmonella enterica,* and we used sodium benzoate, sodium nitrate, and

https://doi.org/10.59720/24-176

Bacteria Type	Preservative Combination	Maximum Allowed Concentration (μg/mL)
Staphylococcus aureus and *Salmonella enterica*	Sodium nitrite + Vitamin E	≤ 230
	Sodium benzoate + Vitamin E	≤ 700
	Sodium benzoate + Sodium nitrite	≤ 1030
	Sodium benzoate + Sodium nitrite + Vitamin E	≤ 1230
Bacillus subtilis	Sodium benzoate + Sodium nitrate + Sodium bisulfite	≤ 1550
	Sodium benzoate + Sodium nitrate	≤ 1030
	Sodium benzoate + Sodium bisulfite	≤ 1500
	Sodium nitrate + Sodium bisulfite	≤ 530

Table 1: Limits for the use of compliant preservatives. Assuming a food concentration of 1 g/mL, these concentrations are the limits for preservatives that are safe under international standards for each bacteria species and preservative combination in this study (36).

Bacteria	Preservative	Concentration (wt%)					
		0.03%	0.06%	0.09%	0.5%	1.0%	1.5%
Staphylococcus aureus	Sodium benzoate	-	-	-	-	-	-
	Sodium nitrite	-	-	-	-	-	-
	Vitamin E	-	-	-	-	-	-
Salmonella enterica	Sodium benzoate	-	-	-	-	-	-
	Sodium nitrite	-	-	-	-	-	-
	Vitamin E	-	-	-	-	-	-
Bacillus subtilis	Sodium benzoate	-	-	-	-	-	-
	Sodium nitrate	-	-	-	-	-	-
	Sodium bisulfite	-	-	-	+	+	+

Table 2: Ability of different preservatives to inhibit bacterial (*Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis*) growth on agar plates under six different concentrations (0.03wt%, 0.06wt%, 0.09wt%, 0.5wt%, 1.0wt%, 1.5wt%). This data represents whether the preservatives had an inhibitory effect on three types of bacteria at six different concentrations. The method used was the inhibition zone technique, where filter papers soaked in the respective preservative were placed evenly in a bacteria plate streaked with each bacteria species. If a clear, measurable inhibition zone appeared, the preservative was determined to have an inhibitory effect. A "+" means there was an antibacterial ring, a "-" means there was no antibacterial ring.

sodium bisulfite to inhibit *Bacillus subtilis*. We measured the antibiotic properties of the preservatives using 2 mm thick filter paper in the diffusion test. We employed the inhibition zone method, where the diameter of the inhibition zone is measured to determine the antibacterial effect. In the inhibition zone experiment, only sodium bisulfite at concentrations of 0.5wt%, 1wt%, and 1.5wt% produced clear and measurable inhibition zones in *Bacillus subtilis*. (**Table 2**). The sizes of the inhibition zones were 2, 2.7, and 3.2 cm, respectively (**Figure 1**). We also performed a bactericidal zone test on sodium benzoate, sodium nitrite, sodium nitrate, and vitamin E, but no significant bacteriostatic zones were measured for these preservatives on any of the tested bacteria (**Table 2**).

We then employed the MIC method to test the growth inhibitory effects of single-variable preservatives on three types of bacteria. We measured the MIC by exposing bacterial cultures to serial dilutions of preservatives, identifying the lowest concentration that prevented visible bacterial growth after 24 hours of incubation. For inhibiting *Staphylococcus aureus*, the MIC of sodium benzoate and sodium nitrite was 0.75wt%. For inhibiting *Salmonella enterica*, the MIC of sodium benzoate was 0.375wt% and the MIC of sodium nitrite was 0.75wt%. For inhibiting *Bacillus subtilis*, the MIC of sodium benzoate was 0.75wt% and the MIC of sodium bisulfite was 0.0375wt% (**Table 3**). We also conducted MIC tests on vitamin E to inhibit *Staphylococcus aureus* and *Salmonella enterica* and on sodium nitrate to inhibit *Bacillus Subtilis*. However, we observed no inhibitory effect at any of the tested concentrations.

Through the univariate experiments, we observed that the antimicrobial efficacy of individual preservatives was low. Consequently, we aimed to explore the combination of multiple preservatives. Given that our inhibition zone and MIC data was qualitative rather than quantitative, we used experimental data from other literature for our optimization model (**Table 4**, 33-35). Linear regression and optimization

https://doi.org/10.59720/24-176

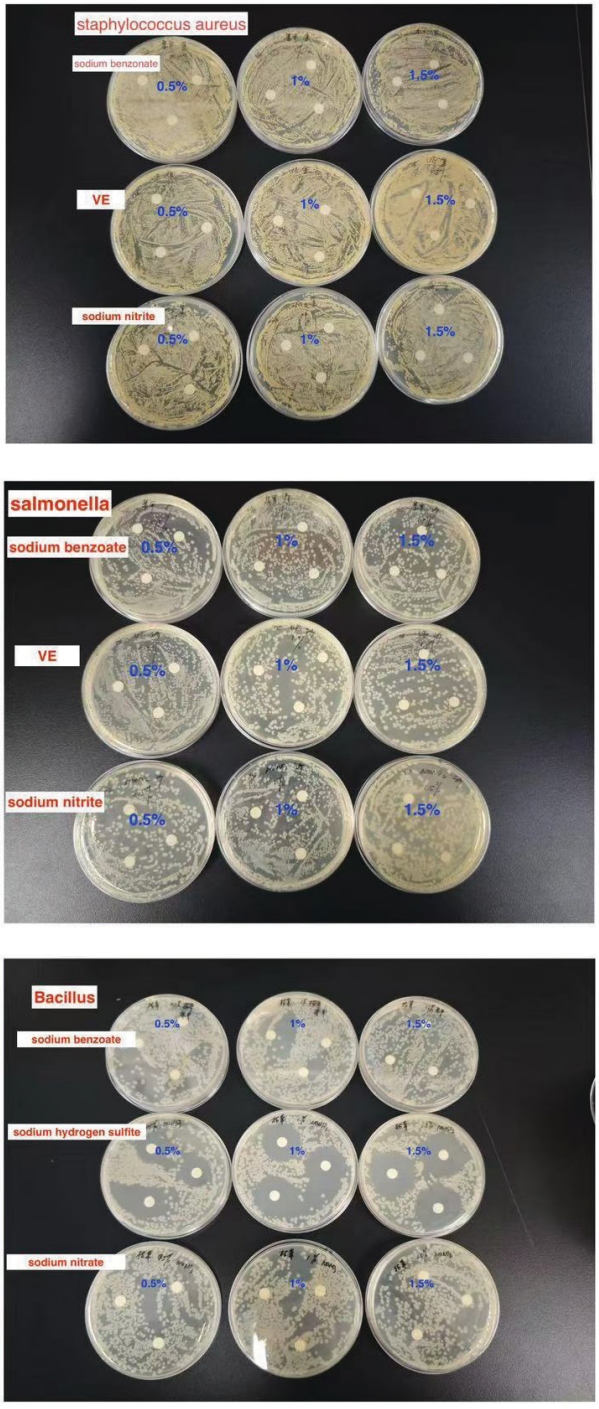

Figure 1: Results of inhibition zone of *Staphylococcus aureus*, *Salmonella* and *Bacillus subtilis* by sodium bisulfite. This figure shows the experimental results using the inhibition zone method. Filter papers (5 mm diameter, 0.1 mm thickness) were sterilized and soaked in various preservative solutions (sodium benzoate, vitamin E, sodium nitrate, sodium nitrite, or sodium bisulfite) at both low (0.03-0.09%) and high (0.5-1.5%) concentrations, then dried at 40 °C. Three bacterial strains (*Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis*) were prepared as 104 CFU/mL suspensions and spread on solid medium plates. The treated filter papers were placed on the inoculated plates, incubated at 28 °C, and observed for 24-72 hours to measure the diameter of inhibition zones. The percentages show the wt% concentrations of each preservative for each dish.

were used to analyze the relationship between preservative concentrations and their inhibitory effects. The regression model fitted a linear equation to the experimental data obtained from literature, while optimization identified the preservative combinations that maximized antimicrobial effectiveness while meeting safety and cost constraints. The purpose of the modeling was to explore the bacterial growth trend under a single preservative and predict the optimal mixing ratio of different preservatives. The goal of linear regression was to find a set of regression coefficients that allow the established linear model to best fit the sample data. Since the amount of data that can be obtained through experiments is limited, this modeling approach can predict more values, allowing us to extrapolate beyond the preservative concentrations we tested experimentally. The linear optimization model is a mathematical programming method that solves for the optimal value of a linear objective function under given constraints. The goal of linear optimization is to solve for the maximum or minimum value of the objective function while satisfying all constraints. This model can help calculate the optimal solution for the blending ratio of preservatives. The objective of our model was to calculate the combination of preservatives that would result in the minimum bacterial growth.

We used univariate data for linear regression and plotted a graph showing the effects of different preservatives on the three bacteria species (*Staphylococcus aureus*, *Salmonella enterica,* and *Bacillus subtilis*). We found that with the increase in preservative concentration, the number of bacteria decreased linearly, and the inhibition effect (the slope of the line) of different preservatives was different. Sodium benzoate showed R-squared values over 0.90 in growth curves of *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis*. The growth curve for *Staphylococcus aureus* with sodium nitrite yielded an R-squared of 0.982. For *Salmonella enterica* treated with Vitamin E, the R-squared value was 1.000 **(Figure 2)**.

After the regression model, we used the linear optimization model to calculate the optimal preservative complex ratio. Sodium nitrate was not included in the model because we were unable to obtain inhibition data for it from literature. The output of this model provided us with specific recommended ratios for combining the tested preservatives to achieve optimal antimicrobial performance. The predicted value was also within the range of preservative usage standards (**Table 1**). Based on these parameters, our optimization model recommended a preservative containing 73.3211 μg/mL sodium benzoate, 11.0424 μg/mL sodium nitrite, 218.958 μg/mL vitamin E, and 12.0742 μg/mL sodium bisulfite to achieve optimal growth inhibition effect and meet the safety standards of preservatives added to food.

It should be noted that the optimal ratio of preservative combination is the concentration value of inhibition of three kinds of bacteria at the same time. Different from the single-variable MIC, the predicted optimal preservative effect aims to be an optimal solution under the three kinds of bacteria and five preservatives selected in this experiment.

DISCUSSION

The overall goal of this study was to test the effect of five preservatives (sodium benzoate, sodium nitrite, vitamin E, sodium nitrate, and sodium bisulfite) against three bacteria

https://doi.org/10.59720/24-176

Bacteria	Preservative	Concentration											MIC (wt%)
		1.5	0.75	0.375	0.187	0.15	0.075	0.037	0.018	0.009	0.004	0.002	
Staphylococcus aureus	Sodium benzoate	+	+	-	-	-	-	-	-	-	-	-	**0.75**
	Sodium nitrite	+	+	-	-	-	-	-	-	-	-	-	**0.75**
	Vitamin E	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella enterica	Sodium benzoate	+	+	+	-	-	-	-	-	-	-	-	**0.375**
	Sodium nitrite	+	+	-	-	-	+	-	-	-	-	-	**0.75**
	Vitamin E	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	Sodium benzoate	+	+	-	-	-	-	-	-	-	-	-	**0.75**
	Sodium nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Sodium bisulfite	+	+	+	+	+	+	+	-	-	-	-	**0.037**

Table 3: The effects of different preservatives on *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis* at eleven concentrations and their minimum inhibitory concentration (MIC). The minimum inhibitory concentration was measured for three types of bacteria using preservatives (1.5wt%) at concentrations of 1.5-0.0023wt%. The lowest concentration that can inhibit bacterial growth is the minimum inhibitory concentration. A "+" means the preservative has an antibacterial effect, while "-" means there is no antibacterial effect.

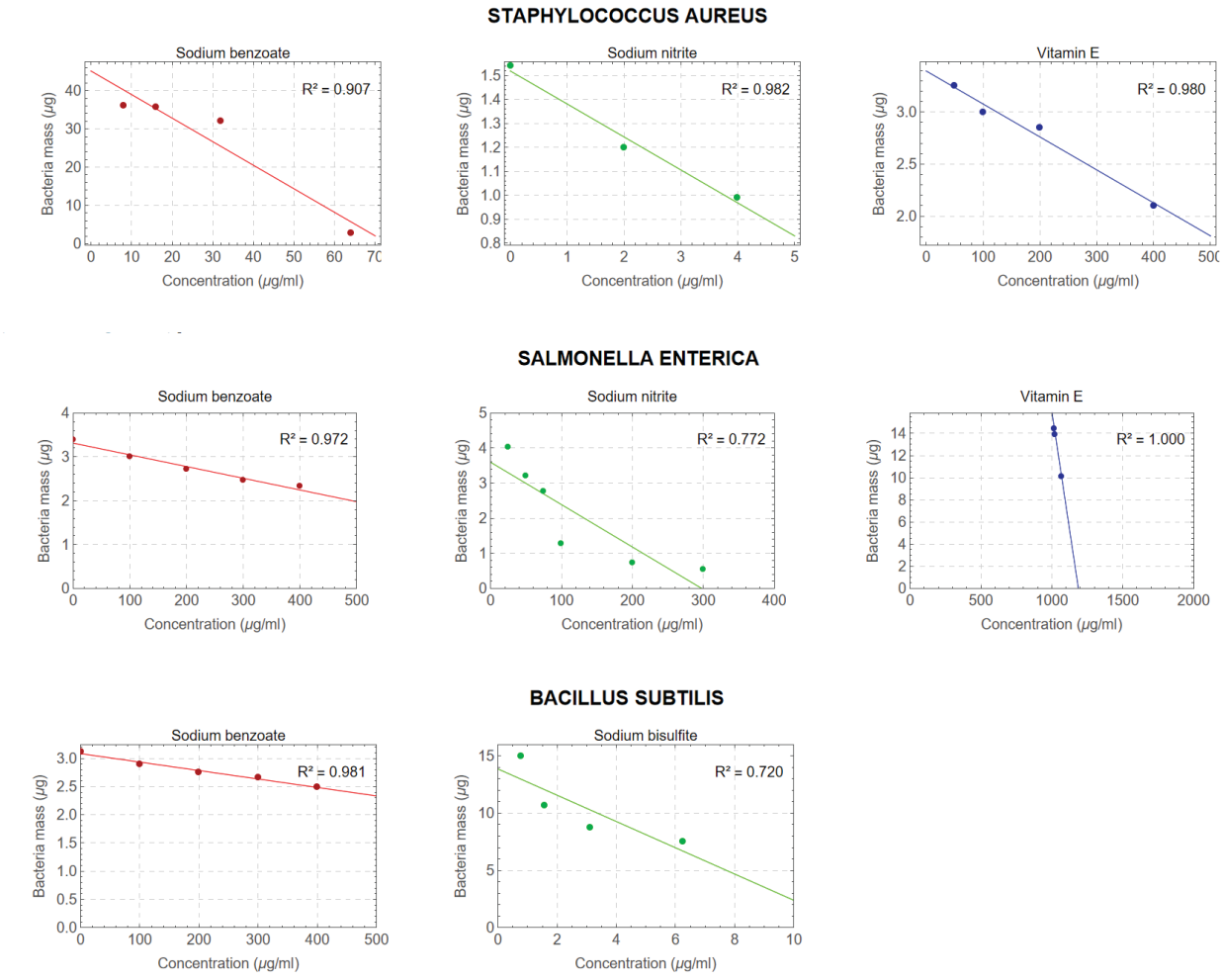

Figure 2: Growth of *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis* in media containing different concentrations of four preservatives based on literature (33-35). The horizontal axis represents the concentration of the preservative (sodium benzoate, sodium nitrite, vitamin E, and sodium bisulfite), while the vertical axis shows the bacteria mass. The bacteria growth was measured using the OD600, which was then used to calculate the equivalent dry weight. Linear regression was performed on each set of datapoints to determine the relationship between the preservative concentration and bacterial growth inhibition.

https://doi.org/10.59720/24-176

Bacteria	Preservative	Concentration (μg/ml)	Bacteria mass (μg)	Reference
Staphylococcus aureus	Sodium benzoate	8	36.00	(Karabay & Sahin, 2005)
		16	35.67	
		32	32.13	
		64	2.80	
	Sodium nitrite	0	1.54	(Bean & Roberts, 1975)
		2	1.2	
		4	0.99	
	Vitamin E	50	3.25	(Al-Salih et al., 2013b)
		100	3.00	
		200	2.85	
		400	2.10	
Salmonella enterica	Sodium benzoate	0	3.39	(Romli et al., 2023)
		100	3.00	
		200	2.71	
		300	2.47	
		400	2.32	
	Sodium nitrite	25	4.03	(De Alba et al., 2013)
		50	3.2	
		75	2.76	
		100	1.27	
		200	0.72	
		300	0.54	
	Vitamin E	1019	14.4	(Ajith et al., 2008)
		1024	13.9	
		1069	10.1	
Bacillus subtilis	Sodium benzoate	0	3.12	(Romli et al., 2023)
		100	2.90	
		200	2.75	
		300	2.66	
		400	2.49	
	Sodium bisulfite	0.781	15.0	(Quoc, 2018a)
		1.563	10.7	
		3.125	8.76	
		6.250	7.53	

Table 4: The number of bacteria of *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis* under different concentrations of preservatives. These data are used to perform linear regression calculations and linear optimization calculations.

https://doi.org/10.59720/24-176

species (*Staphylococcus aureus*, *Salmonella enterica*, *Bacillus subtilis*). We sought to predict the optimal ratio of the concentration of these five preservatives when the three bacteria are present at the same time.

We first tested the growth inhibition of the five preservatives by measuring the zone of inhibition surrounding a preservative-containing filter paper on a petri dish inoculated with each bacteria type. The concentrations used in the inhibitory zone method ranged from 0.5wt% to 1.5wt%. In the experiment, sodium bisulfite demonstrated effective inhibition against *Bacillus subtilis*, with inhibition zones expanding proportionally to increasing concentrations.

The comparison of the inhibitory effect of sodium benzoate on *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis* showed that sodium benzoate was most effective in inhibiting the growth of *Salmonella enterica*. It is known that sodium benzoate's antimicrobial action primarily works by penetrating bacterial cell walls as benzoic acid in acidic conditions (7). It increases cell wall permeability and disrupts internal pH balance. Bacteria attempt to expel the acid using efflux pumps, but this process is energy-intensive (9). The combined effects of cell wall damage, pH disruption, and metabolic interference inhibit bacterial growth. This mechanism may suggest that bacteria with thinner cell walls and fewer efflux pumps may be more susceptible to sodium benzoate. Therefore, we suspect that *Salmonella enterica* was more sensitive due to its thinner cell wall and fewer efflux pumps (10). *Bacillus subtilis* may rely more heavily on aerobic respiration compared to *Salmonella enterica* (11). This could potentially make *Bacillus subtilis* more susceptible to sodium benzoate's possible effects on the respiratory chain (28). As a facultative anaerobe, *Salmonella* can grow both in the presence and absence of oxygen, utilizing different metabolic pathways for energy production depending on oxygen availability (12). Additionally, *Salmonella enterica* possesses a citrate transport system, which could potentially reduce sodium benzoate accumulation, a system that *Bacillus subtilis* appears to lack (13,14). These differences might contribute to the varying sensitivities to sodium benzoate observed between these bacteria, but further research is needed to confirm these hypotheses.

The comparison of the inhibitory effect of vitamin E on *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis* showed that vitamin E alone was ineffective as a preservative. vitamin E's growth inhibition effects vary based on bacterial species, growth stage, and dosage (29). As a safe, natural preservative, it has broad application prospects. Vitamin E can be used effectively as a food preservative in a variety of products through different methods. It is commonly added to cooking oil, vegetable oil, and salad dressings to prevent rancidity, while in meat products it helps preserve color and prevent oxidation (21). This preservative can be added to baked goods such as breads, cookies, and cereals to extend shelf life and maintain nutritional value. Application methods include direct addition during food processing, incorporation into packaging materials, or spraying on food surfaces (26). For best results, vitamin E is often used in combination with other natural antioxidants such as vitamin C, making it a universal solution for natural food preservation (15). Combining vitamin E with other natural preservatives or optimizing its use in food processing, such as with high-pressure processing or ozone disinfection, probably enhances its antibacterial effects and reduces microbial contamination while minimizing adverse effects on food quality (30).

Sodium nitrite demonstrated similar inhibitory effects on both *Staphylococcus aureus* and *Salmonella enterica* at equivalent concentrations. The antimicrobial action of sodium nitrite likely involves the nitrite ion (NO^{2-}) penetrating bacterial cells and binding to cytochrome c oxidase, potentially disrupting electron transfer and inhibiting ATP synthesis (16,17). However, the precise mechanisms and any species-specific differences require further investigation (18,19).

The MIC experiments demonstrated the differential effectiveness of preservatives against three bacterial strains: *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis*. Comparisons with existing literature revealed nuanced variations in preservative efficacy. For *Staphylococcus aureus*, our experimental MICs for sodium benzoate and sodium nitrite were 0.75wt% and 0.75wt%, respectively, which diverge from literature values of 1wt% and 0.1wt% (6). Similarly, for *Bacillus subtilis*, we observed MICs of 0.75wt% for sodium benzoate and 0.0375wt% for sodium bisulfite, closely aligning with literature values of 1wt% and 0.0312wt% (8). While some results deviated from existing research, others showed remarkable consistency.

Although our experiments did not directly test these factors, previous studies have shown that preservative efficacy can be significantly influenced by pH, storage temperature, humidity, and bacterial adaptive mechanisms (25,26). Among these factors, pH and bacterial adaptive responses appear most relevant to our findings, as *Bacillus subtilis* is known to develop resistance through biofilm formation and stress response systems (27). These factors could potentially explain the differences observed in our results, particularly the non-responsiveness of *Staphylococcus aureus* to certain preservative treatments. Potential experimental variables, including reagent preparation and inoculum size, may have also contributed to the observed variations in preservative effectiveness.

Different preservatives have varying antimicrobial effects on bacterial strains, but their mechanisms are similar. A mixture of preservatives is more effective than one, and environmental factors significantly influence the antimicrobial effect (31). The linear optimization model calculated the optimal proportions of the five preservatives for inhibiting growth of the three bacteria species.

To enhance the quality and reliability of future experiments, we propose focusing on two critical environmental variables. First, we suggest adjusting the experimental temperature to match the optimal growth conditions of different bacteria, which can be achieved using programmable incubators. Second, we suggest optimizing the pH value of the medium, as pH significantly affects preservative effectiveness in most case (25). This could be implemented by using buffered media systems. By controlling these key factors, we can improve the robustness of our findings and their real-world applicability. While other factors like oxygen concentration, light conditions, and humidity may also influence results, prioritizing temperature and pH control offers a balanced approach between experimental complexity and meaningful outcomes.

In addition, two key improvements are proposed to strengthen this research. First, expanding our investigation to include natural antimicrobial compounds, particularly plant-

https://doi.org/10.59720/24-176

derived essential oils, would address the growing demand for clean-label preservation. Second, conducting long-term stability studies (6-12 months) would help evaluate potential tolerance development of bacteria to preservatives. This would involve monitoring minimum inhibitory concentrations against target organisms over time, providing valuable data for products with extended shelf lives and helping establish more reliable preservation protocols. These enhancements will help determine optimal preservative combinations and concentrations. Finally, people can create a robust database for building more accurate predictive models of preservative effects.

The selection of preservatives for this study was constrained by available resources and costs. While the chosen preservatives demonstrated inhibitory effects on the bacteria, it is important to note that they are not the only substances capable of such inhibition. Our research focused on a specific range of preservatives within these practical limitations. Future studies with broader scope may identify additional effective preservatives. In all, this study not only provides a better ratio for industrial preservatives but also attempts to predict the compounding ratio by using data modeling, which provides a new method and idea for subsequent research and provides a quick tool for future research on the dosage of natural preservatives.

By comprehensively considering these factors and implementing corresponding research strategies, we expect to be able to develop safer, more effective, and more popular food preservation schemes. This may also lead to breakthroughs in the research of inhibition mechanisms, making important contributions to the development of the food industry.

MATERIALS AND METHODS

Chemicals and Reagents

For this experiment, three species of bacteria were selected: *Staphylococcus aureus* (CMCC(B)26003), *Salmonella enterica typhi* (CMCC(B)50094), and *Bacillus subtilis* (CMCC(B)63501). All three bacterial strains were sourced from the China Medical Culture Collection (CMCC). To cultivate and test these microorganisms, Tryptic Soy Broth (TSB) was used as the liquid growth medium, while Tryptic Soy Agar (TSA) served as the solid growth medium for plating. Sodium benzoate, sodium nitrite, vitamin E, sodium nitrate, sodium bisulfite were all purchased from Becton, Dickinson and Company. The purchased TSA agar was used for the solid plate portion of the experiment and the paper disk diffusion test section, while TSB is a liquid medium used for the MIC determination section. All solutions were prepared with sterile distilled water.

Disk Diffusion Measurement of Antibacterial Zone Method

To prepare the preservative paper for the disk diffusion measurement using the antibacterial zone method, we made circular filter paper pieces with a 5 mm diameter and 0.1 mm thickness using a punch and sterilized them at 121°C for 20 minutes. For the low-concentration experiment, the filter papers were soaked in solutions with concentrations of 0.03%, 0.06%, and 0.09% of sodium benzoate, vitamin E, sodium nitrate, sodium nitrite, or sodium bisulfite until fully absorbed (about forty minutes), with 20 filter papers per group. The filter papers were then placed in a sterile conical tube and dried in a 40°C oven with shaking for about 1 hour. The filter papers were dried for the convenience of preservation. The high-concentration experiment followed the same procedure but with concentrations of 0.5wt%, 1wt%, and 1.5wt% of the preservatives.

Staphylococcus aureus, *Salmonella enterica*, and *Bacillus subtilis* were prepared as bacterial suspensions with a concentration of 104 CFU/mL. A disposable sterile swab was used to coat each solid medium plate with one bacteria strain, with six plates for each type of bacteria (the sixth plate is the negative control). The prepared filter paper pieces were placed at equal distances on the coated plates, which were then incubated at 28°C. Each dish had three pieces of filter paper. The results were observed within 24-72 hours, and the diameter of the antibacterial circle was measured and averaged for each group. The filter paper was cut with a machine, so each filter paper had the same radius.

MIC Method

The preservatives were serially diluted in tryptic soy broth (TSB) to final concentrations of 1.5wt%, 0.75wt%, 0.375wt%, 0.188wt%, and so on. The ability of each preservative to inhibit bacteria growth was determined by visual observation after 24-72 hours. The minimum concentration that could be inhibited was observed and this concentration was recorded as the Minimum Inhibitory Concentration (MIC).

Linear Regression Models and Linear Optimization Model to Calculate the Optimal Mix Ratio

We analyzed data from literature using linear regression and linear optimization models. The linear regression model was based on the data we obtained from literature, since our experimental data was qualitative rather than quantitative (**Table 4**, 33-35). The model was constructed using Wolfram Language to analyze the relationship between preservative concentrations and their antimicrobial effectiveness. Subsequently, we employed a linear optimization model based on the linear regression results to determine the optimal combination of preservatives that would maximize antimicrobial efficacy while minimizing overall preservative concentration.

First, we used the data to implement the linear regression model and calculated the linear equation for the relationship between *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis* growth and different preservative concentrations. We then developed a linear optimization model to identify a mathematical solution for combining preservatives. Considering the use of preservative standards, we adopted the number of preservatives prescribed by national standards as the constraints of the linear optimization model. Preservative concentration limits for *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus subtilis* range from 230 to 1550 μg/mL, with sodium benzoate, sodium nitrate, and sodium bisulfite reaching ≤ 1550 μg/mL for *Bacillus subtilis* and sodium benzoate, sodium nitrite, and vitamin E at ≤ 1230 μg/mL for *Staphylococcus aureus* and *Salmonella enterica* (**Table 1**). We use the sum of the eight groups of linear functions fitted above as the objective function. Since the bacteria mass cannot be negative, all variables were constrained to be non-negative. Finally, a linear optimization model was constructed and solved by the Simplex Method.

https://doi.org/10.59720/24-176

Received: June 20, 2024
Accepted: August 12, 2024
Published: July 6, 2025

REFERENCES

1. Balouiri, Mounyr, Moulay Sadiki, and Saad Koraichi Ibnsouda. "Methods for in vitro evaluating antimicrobial activity: A review." *Journal of pharmaceutical analysis* 6.2 (2016): 71-79. https://doi.org/10.1016/j.jpha.2015.11.005.
2. Hafidh, Rand R., *et al.* "Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product." *The open microbiology journal* 5 (2011): 96. https://doi.org/10.2174/1874285801105010096.
3. Remenant, Benoît, *et al.* "Bacterial spoilers of food: behavior, fitness and functional properties." *Food microbiology* 45 (2015): 45-53. https://doi.org/10.1016/j.fm.2014.03.009.
4. Stanojevic, D., *et al.* "Antimicrobial effects of sodium benzoate, sodium nitrite and potassium sorbate and their synergistic action in vitro." *Bulgarian Journal of Agricultural Science* 15.4 (2009): 307-311.
5. Odeyemi, Olumide Adedokun, *et al.* "Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin." *Comprehensive reviews in food science and food safety* 19.2 (2020): 311-331. https://doi.org/10.1111/1541-4337.12526.
6. Demirkesen, Ilkem, *et al.* "A study on staling characteristics of gluten-free breads prepared with chestnut and rice flours." *Food and bioprocess technology* 7 (2014): 806-820. https://doi.org/10.1007/s11947-013-1099-3.
7. Beuchat, L. R. "Comparison of anti-Vibrio activities of potassium sorbate, sodium benzoate, and glycerol and sucrose esters of fatty acids." *Applied and Environmental Microbiology* 39.6 (1980): 1178-1182. https://doi.org/10.1128/aem.39.6.1178-1182.1980.
8. Finster, Kai. "Microbiological disproportionation of inorganic sulfur compounds." *Journal of Sulfur Chemistry* 29.3-4 (2008): 281-292. https://doi.org/10.1080/17415990802105770.
9. Davidson, P. Michael, John N. Sofos, and A. Larry Branen. *Antimicrobials in food.* CRC press, 2005. https://doi.org/10.1201/9781420028737
10. Pothula, Karunakar Reddy. *Molecular Basis of Gating and Permeation in Bacterial Outer Membrane Channels*. Diss. Jacobs University Bremen, 2017. http://nbn-resolving.org/urn:nbn:de:gbv:579-opus-1007638.
11. Hoffmann, Tamara, *et al.* "The anaerobic life of Bacillus subtilis: cloning of the genes encoding the respiratory nitrate reductase system." *FEMS microbiology letters* 131.2 (1995): 219-225. https://doi.org/10.1111/j.1574-6968.1995.tb07780.x.
12. Hegyi, Anna. "Molecular genetics of spread of Salmonella genomic island 1 (SGI1)." (2018).
13. Prakash, Shraddha, *et al.* "The ion transporter superfamily." *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1618.1 (2003): 79-92. https://doi.org/10.1016/j.bbamem.2003.10.010.
14. Krom, Bastiaan P., *et al.* "Complementary metal ion specificity of the metal-citrate transporters CitM and CitH of Bacillus subtilis." *Journal of bacteriology* 182.22 (2000): 6374-6381. https://doi.org/10.1128/jb.182.22.6374-6381.2000.
15. Taban, Birce Mercanoglu, *et al.* "Value-added effects of using aromatic plants in foods and human therapy." *Food Science and Technology* 42 (2021): e43121. https://doi.org/10.1590/fst.43121.
16. Reddy, D., J. R. Lancaster Jr, and Daren P. Cornforth. "Nitrite inhibition of Clostridium botulinum: electron spin resonance detection of iron-nitric oxide complexes." *Science* 221.4612 (1983): 769-770. https://doi.org/10.1126/science.6308761.
17. Hyduke, Daniel R., *et al.* "Integrated network analysis identifies nitric oxide response networks and dihydroxyacid dehydratase as a crucial target in Escherichia coli." *Proceedings of the National Academy of Sciences* 104.20 (2007): 8484-8489. https://doi.org/10.1073/pnas.0610888104.
18. Müller-Herbst, Stefanie, *et al.* "Identification of genes essential for anaerobic growth of Listeria monocytogenes." Microbiology 160.4 (2014): 752-765. https://doi.org/10.1099/mic.0.075242-0.
19. Honikel, Karl-Otto. "The use and control of nitrate and nitrite for the processing of meat products." *Meat science* 78.1-2 (2008): 68-76. https://doi.org/10.1016/j.meatsci.2007.05.030.
20. Honikel, Karl-Otto. "The use and control of nitrate and nitrite for the processing of meat products." *Meat science* 78.1-2 (2008): 68-76. https://doi.org/10.1016/j.meatsci.2007.05.030.
21. Carocho, Márcio, Patricia Morales, and Isabel CFR Ferreira. "Natural food additives: Quo vadis?." *Trends in food science & technology* 45.2 (2015): 284-295. https://doi.org/10.1016/j.tifs.2015.06.007.
22. Gyawali, Rabin, and Salam A. Ibrahim. "Natural products as antimicrobial agents." *Food control* 46 (2014): 412-429. https://doi.org/10.1016/j.foodcont.2014.05.047.
23. Shaaban, Hamdy A. "Essential oil as antimicrobial agents: Efficacy, stability, and safety issues for food application." *Essential oils-bioactive compounds, new perspectives and applications* (2020): 1-33. https://doi.org/10.5772/intechopen.92305.
24. Lucera, Annalisa, *et al.* "Food applications of natural antimicrobial compounds." *Frontiers in microbiology* 3 (2012): 287. https://doi.org/10.3389/fmicb.2012.00287.
25. Tajkarimi, M. M., Salam A. Ibrahim, and D. O. Cliver. "Antimicrobial herb and spice compounds in food." *Food control* 21.9 (2010): 1199-1218. https://doi.org/10.1016/j.foodcont.2010.02.003.
26. Davidson, P. Michael, T. Matthew Taylor, and Shannon E. Schmidt. "Chemical preservatives and natural antimicrobial compounds." *Food microbiology: fundamentals and frontiers* (2012): 765-801. https://doi.org/10.1128/9781555818463.ch30.
27. Buendía, Laura, *et al.* "Active cardboard box with a coating including essential oils entrapped within cyclodextrins and/or halloysite nanotubes. A case study for fresh tomato storage." *Food Control* 107 (2020): 106763. https://doi.org/10.1016/j.foodcont.2019.106763.
28. Vlamakis, Hera, *et al.* "Sticking together: building a biofilm the Bacillus subtilis way." *Nature Reviews Microbiology* 11.3 (2013): 157-168. https://doi.org/10.1038/nrmicro2960.
29. Brul, Stanley, and Peter Coote. "Preservative agents in foods: mode of action and microbial resistance

https://doi.org/10.59720/24-176

mechanisms." *International journal of food microbiology* 50.1-2 (1999): 1-17. https://doi.org/10.1016/S0168-1605(99)00072-0.
30. Han, Sung Nim, and Simin Nikbin Meydani. "Antioxidants, cytokines, and influenza infection in aged mice and elderly humans." *The Journal of infectious diseases* 182.Supplement_1 (2000): S74-S80. https://doi.org/10.1086/315915.
31. Barba, Francisco J., *et al.* "Mild processing applied to the inactivation of the main foodborne bacterial pathogens: A review." *Trends in Food Science & Technology* 66 (2017): 20-35. https://doi.org/10.1016/j.tifs.2017.05.011.
32. Cui, Haiying, *et al.* "Antibacterial mechanism of oregano essential oil." *Industrial Crops and Products* 139 (2019): 111498. https://doi.org/10.1016/j.indcrop.2019.111498.
33. De Alba, *et al.*, "Combined Effect of Sodium Nitrite With High-pressure Treatments on the Inactivation of Escherichia Coli BW25113 and Listeria Monocytogenes NCTC 11994" https://doi.org/10.1111/lam.12031.
34. Al-Salih, Dalia Abd Al Kader, *et al.* "Antibacterial Effects of Vitamin E: In Vitro Study." *Journal of Biotechnology Research Center,* vol. 7, no. 2, June 2013, pp. 17–23, https://doi.org/10.24126/jobrc.2013.7.2.260.
35. Karabay, O., and I. Sahin. "In Vitro Activity of Sodium-benzoate Against Isolates of Methicillin-resistant Staphylococcus Aureus." *West Indian Medical Journal,* vol. 54, no. 2, Mar. 2005, https://doi.org/10.1590/s0043-31442005000200004.
36. Codex Alimentarius Commission's (CAC) General Standard for Food Additives (GSFA)." Food and Agriculture Organization of the United Nations, https://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/en/.

APPENDIX

The Language used for coding is Wolfram Language, code and models are available at https://github.com/dorisgyl/PresvRatio