BTEC 9200 A and B

Student's Name: Peirong Ren

Student ID: 2197869

Supervisor Name: Harriet Whiley

Project Title: Development of New Food-grade Disinfectant Based on Soybean Isoflavone

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Declaration:

I certify that this thesis does not contain material which has been accepted for the award of any degree or diploma; and to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text of this thesis.

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Abstract

This article explores the development ideas and advantages of food-grade disinfectants based on soy isoflavones (SI). Traditional food-grade disinfectants have shortcomings and problems such as corrosiveness, instability, irritation, and toxicity, which may affect food safety and quality. SI is a natural flavonoid compound with physiological activities such as antioxidant, anti-cancer, anti-inflammatory, and endocrine regulation. At the same time, SI also has antibacterial effects and can inhibit or kill some bacteria, fungi, viruses and other microorganisms. From the perspective of food-grade disinfectants, existing research has not found that SI has adverse effects on human health, does not have the corrosive effect of traditional disinfectants, and can even protect metals and clothing. The authors plan to develop a method that differs from the traditional method of extracting SI from soybeans. At the same time, the author plans to introduce more influencing factors such as temperature, light, and air during the development process. The final experimental results confirmed that SI has good antibacterial properties and stability, which strongly proved that SI has great potential to become a new food-grade disinfectant.

Key words: Soy Isoflavone; Food-grade disinfectants; Antibacterial effects; Stability

Introduction

Foodborne pathogenic bacteria refer to a type of bacteria that cause human diseases using food as a carrier (Bintsis, 2017). This type of bacteria can enter the human body through contaminated water, animal-derived foods, fresh fruits and vegetables, etc., causing varying degrees of illness, including gastrointestinal inflammation, poisoning, sepsis, etc., seriously affecting human health (Priyanka *et al*., 2016). There are many types of foodborne pathogenic bacteria, and different bacteria have different characteristics, transmission routes, and pathogenic mechanisms (Gourama, 2020). At the same time, in addition to posing a threat to human health and life, foodborne pathogenic bacteria also cause huge losses to food production, processing, circulation and consumption (Vikram *et al*., 2022).

Food-grade disinfectants refer to a class of disinfectants that have been tested and certified to disinfect food contact surfaces or equipment (Maillard & Pascoe, 2023). It must comply with the standards and regulations of different countries. These regulations usually include non-toxic or low toxicity to the human body, no or low pollution to food, broad-spectrum and efficient inhibitory effects on microorganisms (Wu *et al*., 2023).

At present, there are many types of food-grade disinfectants commonly used in the market. Almost all these disinfectants have withstood the long-term test of the market and have largely influenced the pattern of the modern food industry (Wirtanen & Salo,

2003). Common food-grade disinfectants include peracetic acid, chlorine dioxide, chlorine-containing disinfectants, quaternary ammonium salt disinfectants, hydrogen peroxide, etc. (Condel *et al*., 2012). They each have their own characteristics and scope of application. In the food industry, practitioners usually need to choose the most suitable disinfectant according to different disinfection purposes and conditions (Stone *et al*., 2020). But it is undeniable that these disinfectants have some shortcomings and problems. For example, most are corrosive to metals and fabrics; most are unstable, easy to decompose or volatilize, and need to avoid high temperature, light, air and other conditions during storage; some are irritating and toxic to the human body, so the workers usually need to wear protective equipment when using them , avoid direct contact of the drug with the skin, mucous membranes and eyes (Zaffora *et al*., 2021; Stone *et al*., 2020). To solve the above problems, it is necessary to develop a food-grade disinfectant that can overcome the above problems.

To overcome the shortcomings of traditional food-grade disinfectants, the author plans to develop a new food-grade disinfectant based on SI. SI are flavonoids widely present in beans and are natural nutritional factors (Pabich & Materska, 2019). Some studies have shown that SI can improve the symptoms of menopausal syndrome in women; prevent and treat osteoporosis; reduce the risk of breast cancer and endometrial cancer in menopausal and postmenopausal women; protect the cardiovascular system, protect arteries, having a certain preventive effect on diseases such as atherosclerosis and coronary heart disease (Pabich & Materska, 2019; Potter, 1998; Zhou *et al*., 2004;

Zheng *et al*., 2016). In addition to many positive effects on human health, SI also have certain antibacterial effects (Dhayakaran *et al*., 2015). SI can inhibit or kill some bacteria, fungi, viruses and other microorganisms; interfere with the metabolism and growth of bacteria, affect the cell wall and membrane structure of bacteria, and destroy the permeability and stability of bacteria; inhibit the toxins and enzymes produced by bacteria (Chen *et al*., 2022; Lalouckova *et al*., 2021). In addition to their benefits for human healthy and antimicrobial properties, SI do not have the corrosive effects of traditional disinfectants on metals and fabrics. Conversely, SI even offer some protection against metals and fabrics (Wan *et al*., 2022; Salman et al., 2022; Deng *et al*., 2019).

Based on the above reasons, the author believes that the food-grade disinfectant developed based on SI could overcomes most of the shortcomings of traditional food-grade disinfectants and has great potential to become a new type of food-grade disinfectant.

Materials and Methods

Raw material and reagents

Soybean powder, Nutrient Broth (OXOID CM0001), Nutrient Agar (OXOID CM0003), *Escherichia coli* (E. coli) (ATCC C-3000), Hexane, Acetone, Ethanol, DMSO (SIGMA), Phosphate buffered saline tablet (PBS) (SIGMA), TWEEN-80

(LABCHEM), Free SI (Genistein) (Shaanxi Zelang Biotech Co., LTD), Sodium Hypochlorite, Benzalkonium Chloride.

Material pretreatment

After crushing the soybeans into powder, put the soybean flour into hexane at room temperature and stir for thirty minutes to degrease.

Organic solvent extraction method to extract SI

According to Yoshiara *et al*. (2012). First, put 500 mg of defatted soybean powder and 25 ml of organic solvent into a 50 ml centrifuge tube. Use water, ethanol, acetone and a solution composed of these three substances for extraction (water: ethanol: acetone = 1:1:1). Extractions were performed for 20, 30, 40, 50, and 60 minutes in a laboratory environment. After the reaction was completed, the mixture in the flask was cooled to room temperature. After the mixture was centrifuged, the supernatant was filtered, placed in a rotary evaporator, and dried under vacuum to obtain SI powder. Figure 1 is a schematic diagram of the organic solvent extraction method.

Figure 1: The main body of the extraction equipment consists of a round-bottomed flask, a reflux condenser tube and a water bath heating device.

Ultrasonic assisted organic solvent extraction method to extract SI

Based on the above extraction method, ultrasonic waves are used to assist the entire reaction. Extractions were performed for 20, 30, 40, 50, and 60 minutes in a laboratory environment. After the extraction is complete, the mixture is centrifuged, and the supernatant is filtered. Then, the supernatant was evaporated using a rotary evaporator to obtain SI powder and weighed. The results were compared with the weight of SI extracted using only organic solvents.

Detect the minimum inhibitory concentration of SI against *E. coli*.

Liquid culture medium for *E. coli* culture

The MIC of SI against *E. coli* was determined using the broth dilution method *E. coli*. colonies were cultured using agar media according to standard methods. After the colonies were formed, an inoculation loop was used to pick 3-5 colonies with similar morphology, inoculated them into 10 ml of broth, and culture them at 37°C for 18-24

hours. After incubation, used a centrifuge to centrifuge the bacterial solution (4000 rpm, 15 min) and removed the supernatant. Resuspend the centrifuged *E. coli*. in PBS and used the pass-through dilution method to dilute the bacterial solution to an optical density of 0.1 at 600 nm (the concentration was $1x10^9$). Use PBS to serially dilute to a concentration of $1x10^4$ and place the bacterial solution into an agar petri dish at each dilution step. After placing the Petri dishes in a 37°C incubator for 24 hours, checked the colonies on the last plate. If the count was approximately 30-300 CFU, the solution was ready for further processing. The schematic diagram of the operating process is shown in Figure 2.

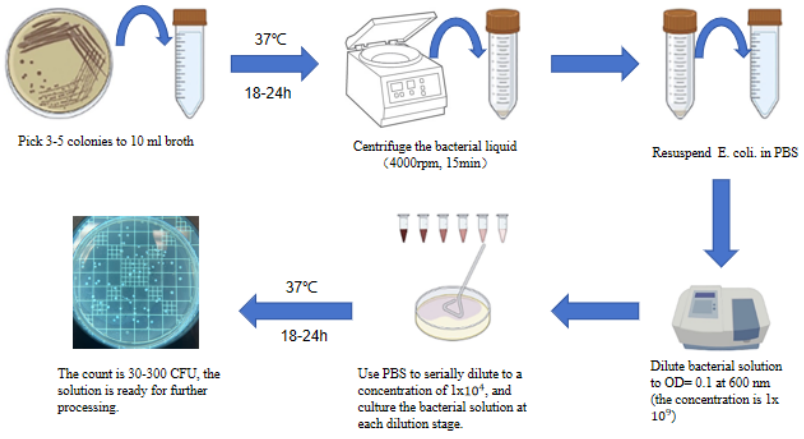


Figure 2. The schematic diagram of the operating process

Determination of MIC by trace broth dilution method

Weigh an appropriate amount of SI powder, dissolve it fully with 100% DMSO, and prepare a storage solution for later use. The concentration of the stock solution is 20mg/ml. The storage solution: Tween-80: PBS = 1:1:18 was mixed to the test solution.

Added 200μL of the test solution with the highest concentration to be measured in well A1, add 100μL PBS in wells A2~A10, then sucked out 100 μL from hole A1 and added it to hole A2, mixed well, then sucked out 100 μL from hole A2 and added it to hole A3, and so on. Analogously diluted the gradient to well A10 and discarded the last 100 μL of the diluted liquid. Added 80 μL nutrient broth (NB) to wells A1-A10. Repeat the above operations for holes B1~B10, C1~C10, and D1~D10. Then added 20 μL PBS to A1~A10 as the negative control group; added 20 μL bacterial solution to B1~B10, C1~C10, and D1~D10 as the test group. Currently, the concentrations of chemicals in A1 to A10 are 500 μg/ml, 250 μg/ml, 125 μg/ml, 63 μg/ml, 32 μg/ml, 16 μg/ml, 8 μg/ml, 4 μg/ml, 2 μg/ml, 1 μg/ml. The concentrations of DMSO are 2.5%, 1.25%, 0.63%, 0.32%, 0.16%, 0.08%, 0.04%, 0.02%, 0.01% and 0.005%.

The DMSO: Tween-80: PBS = 1:1:18 was mixed to the DMSO test solution. Added 100 μl DMSO test solution to well E1, added 100 μl PBS to wells E2 to E10, then sucked out 100 μl from hole E1 and added it to hole E2, mixed well, then sucked out 100 μl from hole E2 and added it to hole E3, and so on for gradient dilution to well E10 and discarded the last 100 μL of diluted liquid. Added 80 μL of NB to wells E1 to E10. Repeat the above operations for F1~F10, G1~G10, H1~H10. Then, 20 μL of PBS was added to E1 to E10 as the negative control group; 20 μL of bacterial liquid was added to F1 to F10, G1 to G10, and H1 to H10 as the test group. At this time, the concentrations of DMSO in the wells were 2.5%, 1.25%, 0.32%, 0.16%, 0.08%, 0.04%, 0.02%, 0.01% and 0.005%.

Add 100 µl water, 80 µl NB and 20 µl bacterial solution to all wells in columns 11 and 12 as a positive control group. Finally, seal the microplate, place it in a plate reader, set the temperature to OD600, 37 degrees Celsius, and incubate for 24 hours. The specific setup of the microplate shown in table 1.

concentration		500μg/ml	250μg/ml	125μg/ml	63μg/ml	32μg/ml	16μg/ml	8μg/ml	4μg/ml	2μg/ml	1μg/ml	PC	
		1	2	3	4	5	6	7	8	9	10	11	12
NC(SI)	A	SI:1000μg/ml 100μl + 80μl NB + 20μl PBS	SI:500μg/ml 100μl + 80μl NB + 20μl PBS	SI:250μg/ml 100μl + 80μl NB + 20μl PBS	SI:125μg/ml 100μl + 80μl NB + 20μl PBS	SI:63μg/ml 100μl + 80μl NB + 20μl PBS	SI:32μg/ml 100μl + 80μl NB + 20μl PBS	SI:16μg/ml 100μl + 80μl NB + 20μl PBS	SI:8μg/ml 100μl + 80μl NB + 20μl PBS	SI:4μg/ml 100μl + 80μl NB + 20μl PBS	SI:2μg/ml 100μl + 80μl NB + 20μl PBS	100μl Deionized water + 80μl NB + 20μl E. coli	
TEST	B	SI:1000μg/ml 100μl + 80μl NB + 20μl E. coli	SI:500μg/ml 100μl + 80μl NB + 20μl E. coli	SI:250μg/ml 100μl + 80μl NB + 20μl E. coli	SI:125μg/ml 100μl + 80μl NB + 20μl E. coli	SI:63μg/ml 100μl + 80μl NB + 20μl E. coli	SI:32μg/ml 100μl + 80μl NB + 20μl E. coli	SI:16μg/ml 100μl + 80μl NB + 20μl E. coli	SI:8μg/ml 100μl + 80μl NB + 20μl E. coli	SI:4μg/ml 100μl + 80μl NB + 20μl E. coli	SI:2μg/ml 100μl + 80μl NB + 20μl E. coli		
	C	SI:1000μg/ml 100μl + 80μl NB + 20μl E. coli	SI:500μg/ml 100μl + 80μl NB + 20μl E. coli	SI:250μg/ml 100μl + 80μl NB + 20μl E. coli	SI:125μg/ml 100μl + 80μl NB + 20μl E. coli	SI:63μg/ml 100μl + 80μl NB + 20μl E. coli	SI:32μg/ml 100μl + 80μl NB + 20μl E. coli	SI:16μg/ml 100μl + 80μl NB + 20μl E. coli	SI:8μg/ml 100μl + 80μl NB + 20μl E. coli	SI:4μg/ml 100μl + 80μl NB + 20μl E. coli	SI:2μg/ml 100μl + 80μl NB + 20μl E. coli		
	D	SI:1000μg/ml 100μl + 80μl NB + 20μl E. coli	SI:500μg/ml 100μl + 80μl NB + 20μl E. coli	SI:250μg/ml 100μl + 80μl NB + 20μl E. coli	SI:125μg/ml 100μl + 80μl NB + 20μl E. coli	SI:63μg/ml 100μl + 80μl NB + 20μl E. coli	SI:32μg/ml 100μl + 80μl NB + 20μl E. coli	SI:16μg/ml 100μl + 80μl NB + 20μl E. coli	SI:8μg/ml 100μl + 80μl NB + 20μl E. coli	SI:4μg/ml 100μl + 80μl NB + 20μl E. coli	SI:2μg/ml 100μl + 80μl NB + 20μl E. coli		
NC(DMSO)	E	DMSO:5% 100μl + 80μl NB + 20μl PBS	DMSO:2.5% 100μl + 80μl NB + 20μl PBS	DMSO:1.25% 100μl + 80μl NB + 20μl PBS	DMSO:0.63% 100μl + 80μl NB + 20μl PBS	DMSO:0.32% 100μl + 80μl NB + 20μl PBS	DMSO:0.16% 100μl + 80μl NB + 20μl PBS	DMSO:0.08% 100μl + 80μl NB + 20μl PBS	DMSO:0.04% 100μl + 80μl NB + 20μl PBS	DMSO:0.02% 100μl + 80μl NB + 20μl PBS	DMSO:0.01% 100μl + 80μl NB + 20μl PBS		
TEST	F	DMSO:5% 100μl + 80μl NB + 20μl E. coli	DMSO:2.5% 100μl + 80μl NB + 20μl E. coli	DMSO:1.25% 100μl + 80μl NB + 20μl E. coli	DMSO:0.63% 100μl + 80μl NB + 20μl E. coli	DMSO:0.32% 100μl + 80μl NB + 20μl E. coli	DMSO:0.16% 100μl + 80μl NB + 20μl E. coli	DMSO:0.08% 100μl + 80μl NB + 20μl E. coli	DMSO:0.04% 100μl + 80μl NB + 20μl E. coli	DMSO:0.02% 100μl + 80μl NB + 20μl E. coli	DMSO:0.01% 100μl + 80μl NB + 20μl E. coli		
	G	DMSO:5% 100μl + 80μl NB + 20μl E. coli	DMSO:2.5% 100μl + 80μl NB + 20μl E. coli	DMSO:1.25% 100μl + 80μl NB + 20μl E. coli	DMSO:0.63% 100μl + 80μl NB + 20μl E. coli	DMSO:0.32% 100μl + 80μl NB + 20μl E. coli	DMSO:0.16% 100μl + 80μl NB + 20μl E. coli	DMSO:0.08% 100μl + 80μl NB + 20μl E. coli	DMSO:0.04% 100μl + 80μl NB + 20μl E. coli	DMSO:0.02% 100μl + 80μl NB + 20μl E. coli	DMSO:0.01% 100μl + 80μl NB + 20μl E. coli		
	H	DMSO:5% 100μl + 80μl NB + 20μl E. coli	DMSO:2.5% 100μl + 80μl NB + 20μl E. coli	DMSO:1.25% 100μl + 80μl NB + 20μl E. coli	DMSO:0.63% 100μl + 80μl NB + 20μl E. coli	DMSO:0.32% 100μl + 80μl NB + 20μl E. coli	DMSO:0.16% 100μl + 80μl NB + 20μl E. coli	DMSO:0.08% 100μl + 80μl NB + 20μl E. coli	DMSO:0.04% 100μl + 80μl NB + 20μl E. coli	DMSO:0.02% 100μl + 80μl NB + 20μl E. coli	DMSO:0.01% 100μl + 80μl NB + 20μl E. coli		

Table 1. The specific setup of the microplate

Comparative Test

The MIC of several common food-grade disinfectants were determined using the broth microdilution method, and the analytical results were compared with the MIC of SI. Understand the advantages and limitations of SI as a naturally derived antimicrobial substance by comparing various properties of SI with selected food-grade disinfectants. Sodium Hypochlorite and Benzalkonium Chloride were selected as comparison objects

in this study. The overall operation process is the same as when detecting the MIC of SI to *E. coli*.

Stability Test

The MIC of SI was obtained from previous experiments. On this basis, the broth dilution method was used to verify the influence of common environmental factors such as temperature, light, and air on the antibacterial performance of SI.

According to Wally-Vallim *et al*. (2014), temperature will affect the biological activity of SI, so the temperature factor was added to the experiment to analyze the biological activity of SI at different temperatures. The SI solutions with the lowest inhibitory concentration were heated at water bath temperatures of 25, 40, 55, 70, 85 and 100 degrees Celsius for 30 minutes. After heating was completed, cool to room temperature and store in a sealed container away from light. SI solutions heated at different temperatures were added to wells A1 to 6, and wells 7 and 8 well were used as negative control groups and positive control groups respectively, and 3 groups were repeated. Place the sealed microplate into a 37°C incubator and judge the results after 24 hours of incubation. Study the effect of temperature on the antibacterial properties of SI.

From the study of Migues *et al*. (2022), ultraviolet rays have a certain degradation effect on flavonoids, so ultraviolet rays were added to the experiment to analyze the biological activity of SI after irradiation by natural light. Under room temperature conditions, the

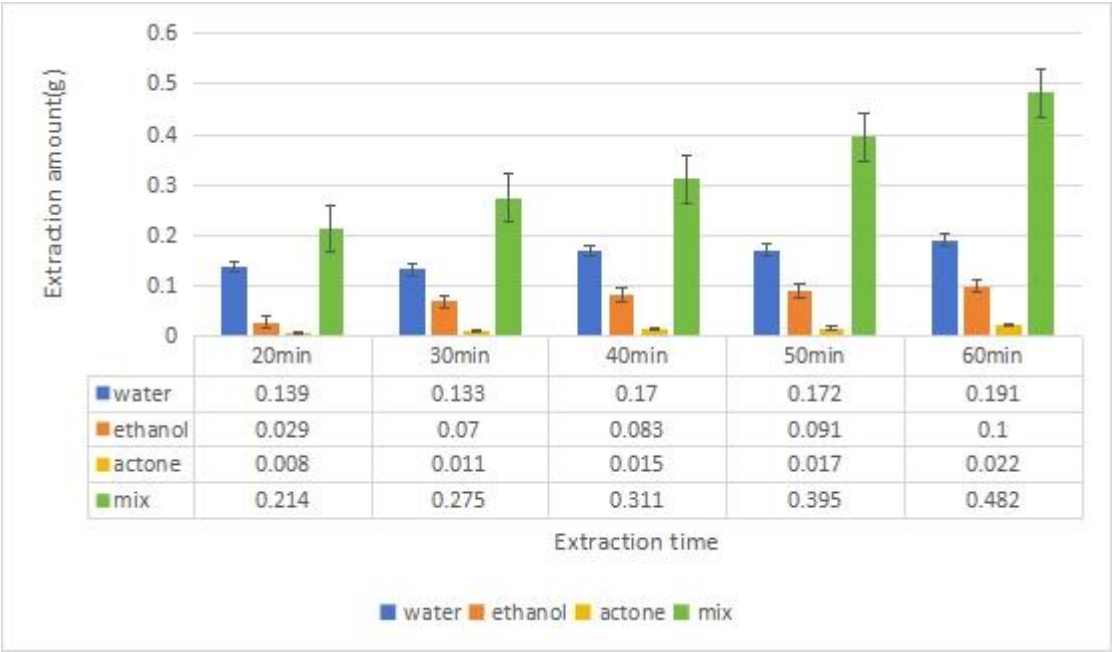
SI powder sealed in a transparent vacuum bag was irradiated with UV lamp for 30, 60, 90, 120, 150 and 180 minutes respectively. After the irradiation is completed, the vacuum bag containing the sample is stored in a dark place. Use the treated SI to prepare a solution with the lowest inhibitory concentration. Add SI solution that has been irradiated with ultraviolet light for different times in wells A1 to 6. Wells 7 and 8 well as the negative control group and the positive control group respectively. Repeat 3 groups. Place the sealed microplate into a 37°C incubator and judge the results after 24 hours of incubation. Study the effect of ultraviolet light on the antibacterial properties of SI.

According to Kathuria and Dhiman's study on SI packaging (2022), whether SI is in contact with air may affect the antibacterial ability of SI to a certain extent, so it is planned to introduce the time factor of contact with air in the experiment. At room temperature, place the unsealed SI powder in a light-proof and ventilated environment to ensure that it is fully in contact with the air. The contact time with air was 30, 60, 90, 120, 150 and 180 minutes respectively. When the contact time is over, put the SI powder back into the vacuum bag, seal it and store it under dark conditions. Use the treated SI to prepare a solution with the lowest inhibitory concentration. Add SI solutions that have been in contact with air for different times in holes A1 to 6. Wells 7 and 8 well as the negative control group and the positive control group respectively. Repeat 3 groups. Place the sealed microplate into a 37°C incubator and judge the results after 24 hours of incubation. Study the effect of normal air on the antimicrobial properties of SI.

Results

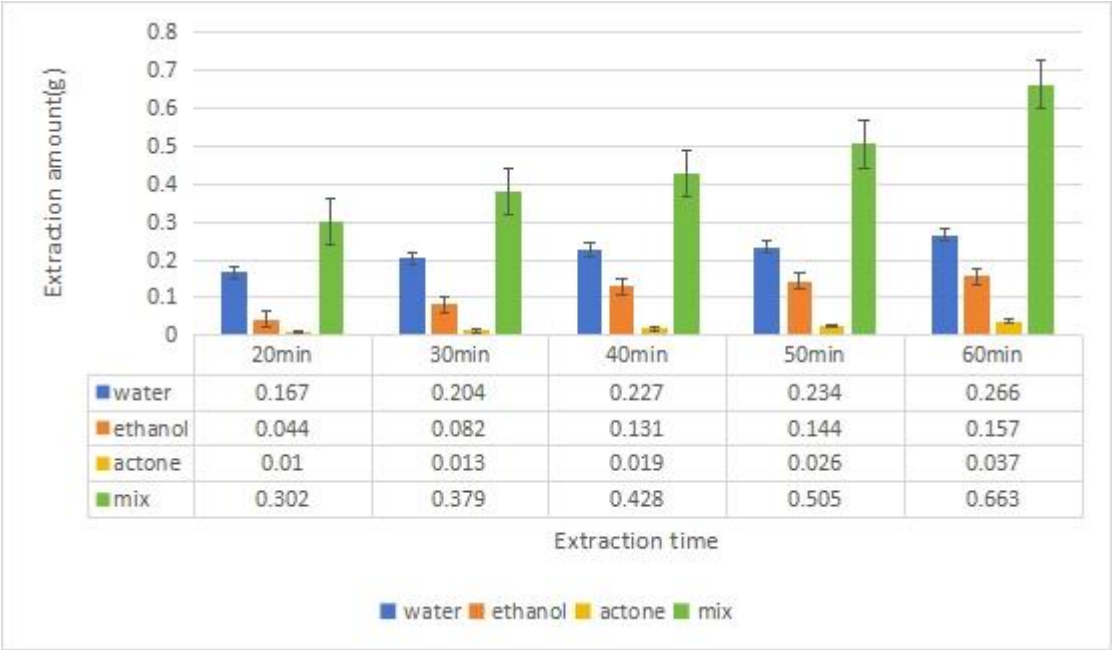
The extraction rate of SI

Through comparison of experimental results, it was found that the extraction effect of SI from defatted soybean flour using a solvent mixed in equal proportions is better than that of a single solvent. On this basis, the use of ultrasonic assistance can significantly improve the extraction efficiency. Under the same extraction time, the yield can be increased by 35% to 48%. Please refer to Table 2&3 for details.



	20min	30min	40min	50min	60min
water	0.139	0.133	0.17	0.172	0.191
ethanol	0.029	0.07	0.083	0.091	0.1
actone	0.008	0.011	0.015	0.017	0.022
mix	0.214	0.275	0.311	0.395	0.482

Table 2. Amount of SI extracted by different solvents at different times.



	20min	30min	40min	50min	60min
water	0.167	0.204	0.227	0.234	0.266
ethanol	0.044	0.082	0.131	0.144	0.157
actone	0.01	0.013	0.019	0.026	0.037
mix	0.302	0.379	0.428	0.505	0.663

Table 3. The amount of SI extracted by different solvents at different times with the assistance of ultrasound.

SI MIC

After analyzing the experimental results of the microbroth dilution test for SI against E. coli, it was found that the MIC of SI against E. coli was 8 μg/ml. At the same time, the growth of E. coli was not inhibited in the control group with the same concentration of DMSO. It can be concluded that DMSO in the SI solution has no effect on the MIC of SI against E. coli. 24-hour growth curve of E. coli in in SI solutions with different concentrations have been shown in Table 4.

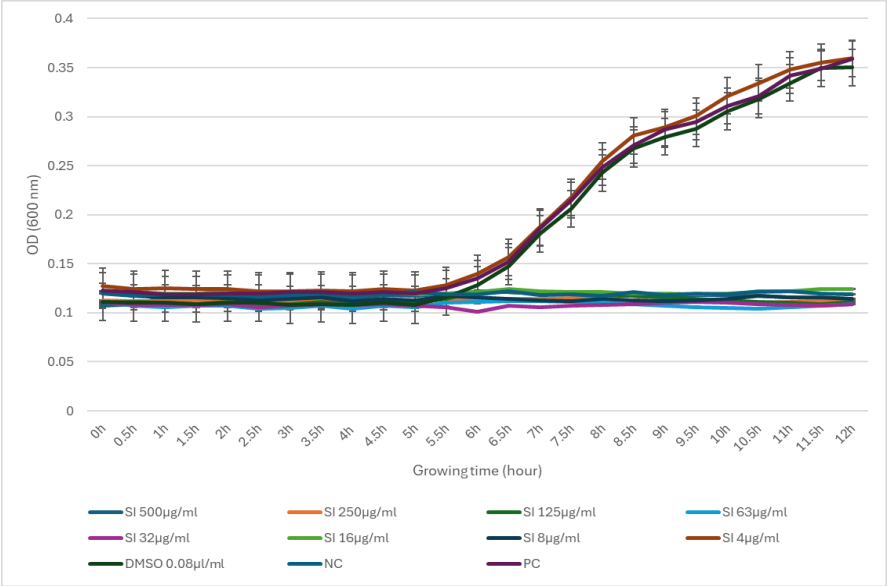


Table 4. Growth curve of E. coli in SI solutions with different concentrations within 24 hours.

Sodium Hypochlorite MIC

It can be seen from the experimental results that the MIC of hypochlorite against E. coli is 63 µg/ml. This result shows that the bacteriostatic property of hypochlorite is lower than that of SI. 24-hour growth curve of E. coli in sodium hypochlorite solutions with different concentrations have been shown in Table 5.

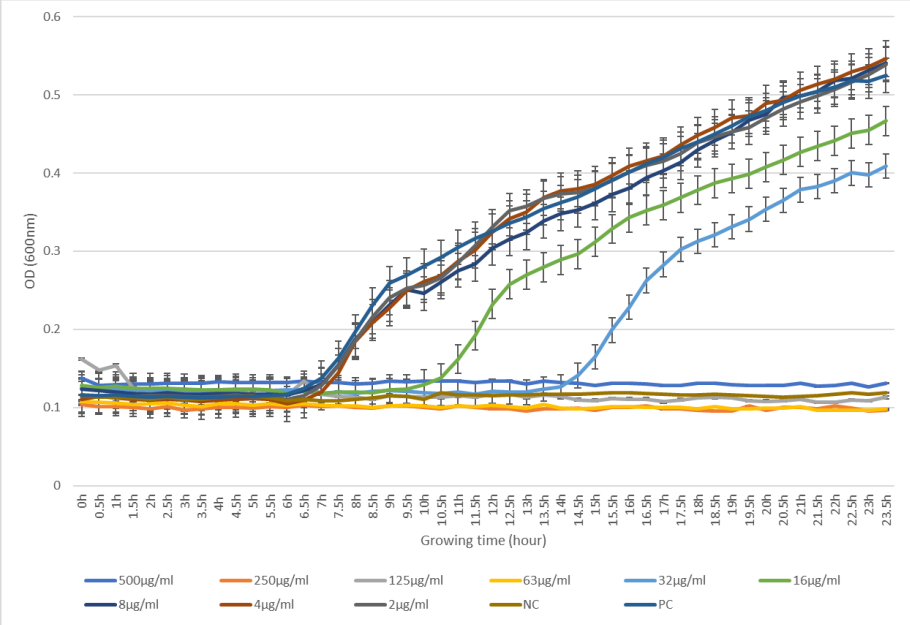


Table 5. Growth curve of E. coli in Sodium Hypochlorite solutions with different concentrations within 24 hours.

Benzalkonium Chloride MIC

By analyzing the experimental results, it was concluded that the MIC of Benzalkonium Chloride against E. coli was 6.3 μg/ml. Compared with SI, its antibacterial ability is better than SI. Growth curve of E. coli in Benzalkonium Chloride solutions with different concentrations within 24 hours have been shown in Table 6.

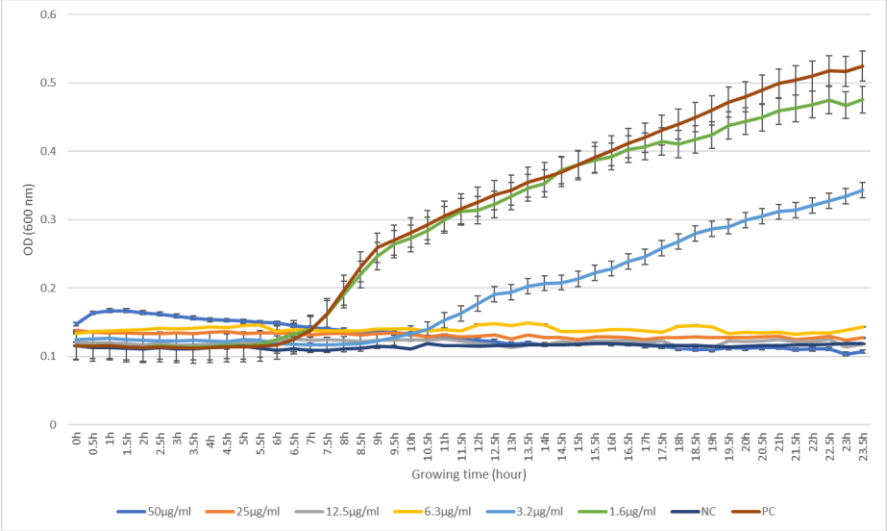


Table 6. Growth curve of E. coli in Benzalkonium Chloride solutions with different concentrations within 24 hours.

Temperature Effect

By analyzing the experimental results, temperature has an impact on the antibacterial activity of SI. SI loses its antibacterial activity when the temperature is above 70℃. The growth curves of E. coli in SI solutions heated at different temperatures within 24 hours are shown in Table 7.

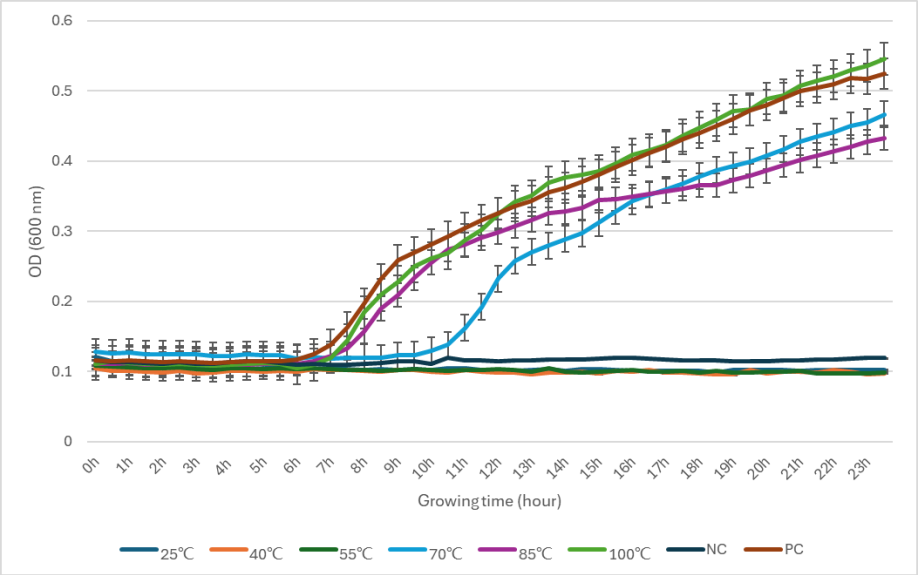


Table 7. Growth curve of E. coli in SI solution heated at different temperatures.

UV Light Effect

Through the analysis of experimental results, no weakening of the antibacterial properties of SI was observed after the selected UV light irradiation time. According to the experimental results, SI can withstand at least three hours of UV irradiation. The 24-hour growth curves of *E. coli* in SI solutions that have experienced different UV irradiation times are shown in Table 8.

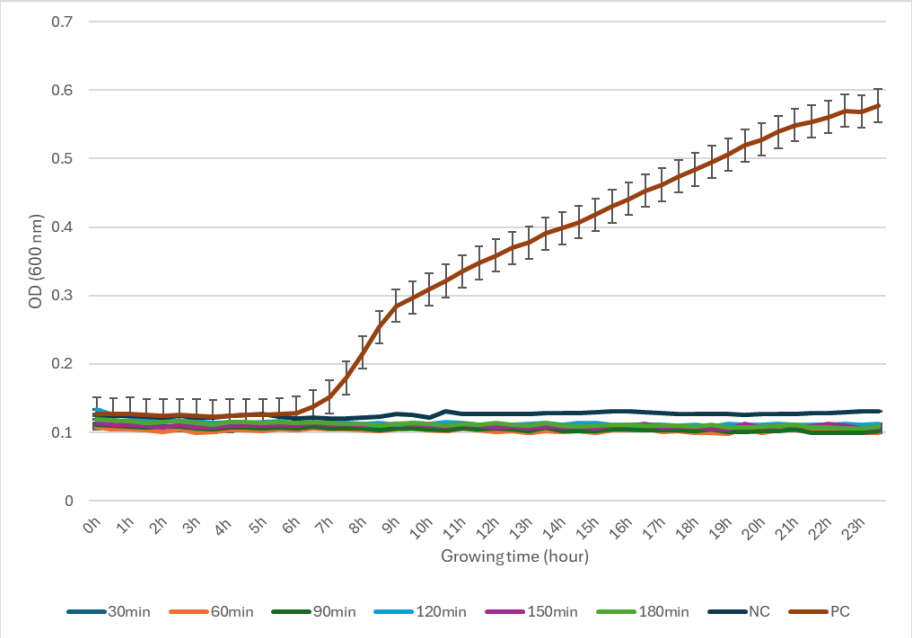


Table 8. Growth curve of E. coli in SI solution experienced different UV irradiation times.

Air Effect

Through analysis of the experimental results, no weakening of the antibacterial properties of SI was observed after the selected air contact time. According to experimental results, SI can remain in contact with air for at least three hours. The 24-hour growth curves of E. coli in SI solutions exposed to air for different times are shown in Table 9.

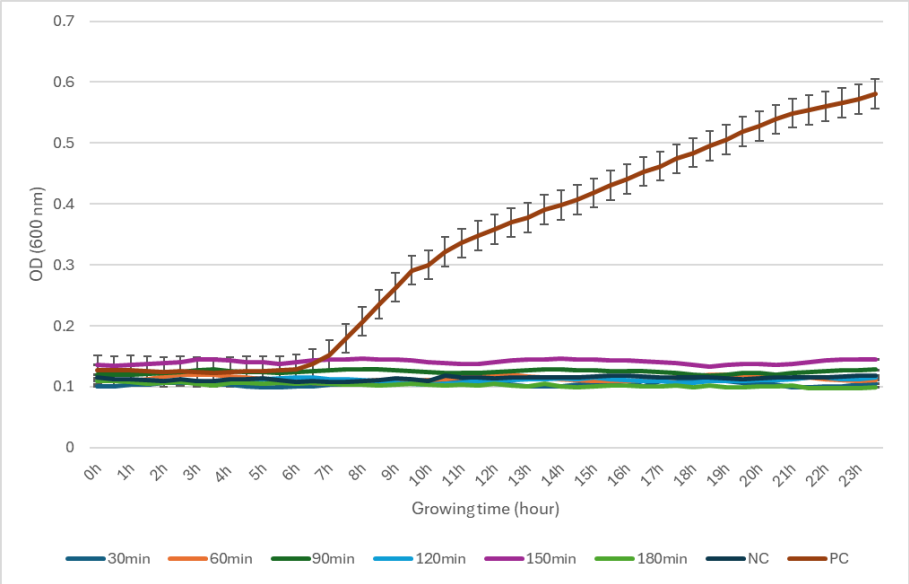


Table 9. Growth curve of E. coli in SI solution exposed to air for different times.

Discussion

By comparing the experimental results, it was found that the effect of using a mixed solvent in equal proportions to extract SI from defatted soybean flour was better than that of a single solvent. On this basis, ultrasonic assistance can significantly improve extraction efficiency. Under the same extraction time, the yield can be increased by 35% to 48%.

In comparative experiments with other disinfectants, it was found that the antibacterial ability of SI was between two common disinfectants. The MIC of SI against E. coli is 8 μg/ml, the MIC of Sodium Hypochlorite is 63 μg/ml, and the MIC of Benzalkonium Chloride is 6.3 μg/ml.

In stability tests, results showed that SI maintained its antimicrobial properties at temperatures of 25-70 degrees Celsius. At the same time, after three hours of ultraviolet irradiation and three hours of air contact, the antibacterial properties of SI were not affected. After analyzing these three results, it was concluded that the stability of SI is excellent.

Combined with the above experimental results, SI has great potential to become a new food-grade disinfectant. At the same time, it also has some advantages that traditional disinfectants do not have. According to existing research on SI, as a natural extract, SI has no toxic side effects on the human body other than allergic reactions, and has no obvious pungent odor or conspicuous color, which means that SI can be used as a food additive.

In addition, SI also has some disadvantages as a food-grade disinfectant. The first problem is the price. The price of SI is more expensive than traditional disinfectants, which will increase the cost of use for users. Secondly, free SI, as an active substance with antibacterial effects, is difficult to dissolve in water and requires the use of organic solvents for dissolution and surfactants to prevent flocculent precipitation during the dilution process, which increases the difficulty of use to a certain extent. In the stability test, the conditions preset by this research are closer to daily use. Except for the heating

test, the other two items did not reach the physical limit of SI and require follow-up research.

Overall, this study achieved the preset goals. By testing the antibacterial ability and stability of SI in different environments, it was proved that SI has good potential to become a new food-grade disinfectant.

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