

Article

The Design and Process Parameters for the Optimization of an Ultrasonic—Thermal Co-Sterilization System for Liquid Eggs

Wei Fan ¹ , Hao Cui ¹, Hong Lu ^{2,*} , Lanqi Meng ² and Qiaohua Wang ¹ 

¹ College of Engineering, Huazhong Agricultural University, Wuhan 430070, China; fanwei@mail.hzau.edu.cn (W.F.); cuihao@webmail.hzau.edu.cn (H.C.); wqh@mail.hzau.edu.cn (Q.W.)

² School of Mechanical and Electronic Engineering, Wuhan University of Technology, Wuhan 430070, China; menglq214@163.com

* Correspondence: landzh@whut.edu.cn; Tel.: +86-136-6723-5418

Abstract: The sterilization of liquid eggs plays a crucial role in the production of liquid egg products. Traditional pasteurization techniques can easily cause protein denaturation, while non-thermal sterilization techniques are often constrained by processing intensity and time. Improving the effectiveness of liquid egg sterilization while preserving the stability of its functional attributes poses a significant challenge. In response to this issue, a synergistic ultrasonic mild thermal sterilization system for liquid eggs is proposed, accompanied by the optimization of its process parameters. COMSOL is employed to simulate the acoustic field distribution of the ultrasonic–thermal system in the liquid egg medium. Verification is conducted through acoustic intensity measurements, and analysis is performed to obtain the optimal arrangement of ultrasonic transducers. Based on Modbus communication, an ultrasonic–thermal synergistic sterilization system is designed. Sterilization experiments are conducted with both 20 kHz + 28 kHz and 20 kHz + 40 kHz multifrequency ultrasound, compared with traditional 20 kHz single-frequency ultrasound. The results indicate that multifrequency ultrasound improves sterilization efficiency by approximately 15% compared to traditional single-source ultrasound. Utilizing multifrequency ultrasonic–thermal synergistic sterilization experiments, a three-factor, three-level response surface test is conducted with sterilization rate and foaming properties as evaluation criteria. The results indicate a strong correlation between ultrasonic frequency, processing time, heating temperature, and sterilization performance, with the impact magnitude being sterilization temperature > processing time > ultrasound frequency. Parameter optimization analysis is performed using a genetic algorithm, yielding sterilization conditions of 55 °C, 11 min and 30 s processing time, and 20 + 40 kHz ultrasonic frequency. The liquid egg sterilization rate is 99.32%, an average decimal reduction of 3.17 log values, and foaming properties are 42.79%. Through comparative analysis, it is determined that the sterilization rate of the ultrasonic–thermal synergistic sterilization system meets national standards, and functional properties such as foaming are superior to traditional pasteurization. This validates the proposed ultrasonic–thermal synergistic liquid egg sterilization control system as effective and feasible.

Keywords: liquid egg; ultrasonic–thermal synergy; response surface analysis; genetic algorithm; process parameter optimization



Citation: Fan, W.; Cui, H.; Lu, H.; Meng, L.; Wang, Q. The Design and Process Parameters for the Optimization of an Ultrasonic—Thermal Co-Sterilization System for Liquid Eggs. *Agriculture* **2024**, *14*, 509. <https://doi.org/10.3390/agriculture14040509>

Academic Editor: Isabel Lara

Received: 4 February 2024

Revised: 2 March 2024

Accepted: 20 March 2024

Published: 22 March 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Liquid egg is a significant research focus in the deep processing of eggs and the food industry [1]. Moreover, the utilization of raw materials in liquid eggs in the food industry can be applied to the production of various foods, such as pastries, meat products, and dairy products. In the baking industry, the utilization of egg whites and egg yolks in whole egg liquid contributes to the formation of food structure. In particular, the foaming properties of whole egg liquid serve as a leavening agent in the food industry, enhancing and maintaining the quality of aerated foods, such as cakes and cookies [2]. Liquid egg

mainly includes liquid whole egg, liquid egg white, liquid egg yolk, and special egg solutions with added salt or sugar. Compared to shell eggs, liquid egg products can retain all the nutritional characteristics of eggs and effectively address issues such as the fragility, transportation, and storage difficulties of fresh shell eggs [3,4]. Liquid egg processing involves removing natural defense barriers such as the eggshell and membrane, making it susceptible to microbial growth during various production processes [5], hence the necessity for sterilization to ensure safety. On this basis, further improving the functionality and nutritional characteristics of liquid egg products is also a research focus [6].

Liquid egg products are highly heat-sensitive, with protein coagulation temperatures of 62–64 °C for egg whites and 68–71.15 °C for egg yolks [7]. The critical challenge in liquid egg production is how to ensure sterilization intensity without compromising the functional properties and nutritional value of liquid egg products. In the United States, Japan, Europe, and Australia, the predominant intervention to enhance the microbial safety of liquid eggs in the egg industry is pasteurization [8]. Pasteurization processing of liquid eggs accounts for 30–40% of egg production [9]. Due to the difficulty of controlling the temperature of pasteurization, ensuring the efficiency of sterilization becomes challenging. If the pasteurization temperature is too low, insufficient sterilization may lead to product spoilage, while excessively high temperatures can adversely affect the functional and sensory properties of eggs. Moreover, different countries have slightly varied requirements for pasteurization temperature and time [10]. In order to minimize the adverse effects of pasteurization on liquid eggs, various new processing techniques have been extensively explored. For example, Geveke et al. [11] utilized radiofrequency (RF) heating and pasteurization to process liquid whole eggs, resulting in a reduction of over 6 logarithmic units in the quantity of *Escherichia coli* and a similar decrease in the count of *Escherichia coli*. Studies by Yan et al. [12] found that super-high pressure sterilization conditions at 300–450 MPa for 15 min were necessary to reduce pathogenic bacteria, such as *Listeria monocytogenes* and *Salmonella*, in egg products to 2 logarithmic units. However, pressures exceeding 300 Mpa led to increased protein viscosity, denaturation, and aggregation. Hermawan et al. [13] demonstrated the sterilization effectiveness of pulsed electric field (PEF) technology on *Salmonella* in liquid whole eggs. Yuk and Geveke [14] found that the extent of sublethal injury in surviving *Lactobacillus plantarum* cells increased with the rise in CO₂ concentration and processing temperature. Under high-pressure CO₂ sterilization conditions of 5 Mpa, 25 °C, for 20–30 min, the sublethal proportion of *Escherichia coli* sharply increased to 72.4%. Furthermore, with an extension of processing time to 60 min, the sublethal proportion of *Escherichia coli* significantly rose to 100%. Some researchers have also found that, for most pathogenic bacteria, combination therapy may be more effective than individual treatment. Compared to traditional pasteurization methods, these novel processing techniques excel in eliminating foodborne pathogens and maintaining the nutrition and quality of liquid eggs.

Ultrasonic technology, as a typical non-thermal sterilization method, can inactivate microorganisms through mechanisms such as cavitation, local heating, and mechanical vibration. It has been widely applied for sterilizing heat-sensitive foods such as milk and beverages [15]. Scholars such as Cao et al. [16] conducted ultrasonic sterilization treatment on barley grass, demonstrating that ultrasonic sterilization treatment can effectively enhance its microbial safety, sensory quality, and storage stability. Research has found that the individual sterilization effect of ultrasound is limited, especially against spores and fungi with strong resistance, resulting in a moderate sterilization effect and significant impact on food quality. The U.S. Food and Drug Administration also does not recommend using single ultrasound as the sterilization process in food production [17]. However, ultrasound in combination with other sterilization methods can effectively reduce processing time, enhance sterilization effectiveness, and, at the same time, maximally preserve the active ingredients in food. For instance, researchers such as Manas [18] studied the synergy of 20 kHz ultrasound with high pressure. With a constant ultrasound frequency and an increase in pressure from 0 to 200 kPa, the D-value (time required for a 90% reduction in

bacterial population) in the test buffer decreased from 5.7 min to 2.5 min. Huang et al. [19] studied the effects of pulsed electric field, hydraulic high pressure, and ultrasonic treatment alone, and in combination, on the inactivation of *Salmonella* in liquid eggs. However, due to cost and efficiency considerations, these methods have not been widely adopted on a large scale. Scholars such as Li et al. [20] investigated the simultaneous damage to membranes and the inhibition of esterase during ultrasonic treatment, resulting in a direct lethal effect. Bacteria under 55 °C heating conditions were inactivated through lethal damage. Scholars such as Lee et al. [21], through the development of an ultrasonic sterilization device, discovered that the use of single-frequency ultrasonication combined with heat can effectively sterilize while preserving the sensory qualities of food. Therefore, the combination of low-frequency, high-intensity ultrasonic waves with mild heat in a synergistic manner, forming a dual-barrier factor, can reduce the requirements on ultrasonic equipment and heat treatment intensity. This not only ensures the functional properties of liquid eggs but also further enhances sterilization efficiency. Li et al. [22] combined the application of ultrasound and microwaves in food processing, where microwaves accelerate heating rates, and ultrasound improves the efficiency of heat and mass transfer. The synergy of the heating effect of microwaves and the cavitation effect of ultrasound enhances processing efficiency and disrupts the cellular structure of materials. Therefore, the above studies indicate that the synergistic use of ultrasound with other sterilization methods yields better results than individual sterilization.

This paper focuses on the design and implementation of a sterilization system using ultrasound as the primary means, supplemented by thermal effects. A Modbus-based ultrasound–thermal synergistic sterilization system is constructed. The simulation of ultrasound field distribution is conducted using COMSOL. Single-factor and multi-factor experiments are designed for ultrasound frequency, temperature, and processing time for liquid egg sterilization. A response surface is employed to explore the comprehensive impact of process parameters on liquid egg sterilization efficiency and foaming properties. The NSGA2 genetic algorithm is used to obtain optimal process parameters, and sterilization experiments are conducted for validation.

2. Materials and Methods

2.1. The Ultrasonic–Thermal Synergistic Sterilization System Proposal

The ultrasonic–thermal synergistic hardware system comprises an ultrasonic system and a constant temperature water bath system. Two ultrasonic generators supporting RS485 communication are connected to four ultrasonic transducers, forming a multifrequency ultrasonic system. The constant temperature water bath system consists of a temperature controller, a heater, and an AC contactor. Coordinated control of the ultrasonic–thermal dual system is carried out in the upper computer software based on the Modbus-RTU communication protocol. The ultrasonic generator and temperature controller are directly powered by a 220 V power supply, while the heating rod and temperature sensor are powered and operated through a low-voltage power supply. A USB/RS485 converter connects the ultrasonic–thermal synergistic control system to the upper computer for data transmission. The general experimental process is as follows: firstly, prepare liquid whole egg. After processing with the ultrasonic–thermal synergistic sterilization system, conduct sterilization rate and functional property testing on the treated egg liquid. Finally, the experimental results indicate that the ultrasonic–thermal synergistic sterilization system exhibits good sterilization effectiveness for liquid eggs. The overall scheme of the ultrasonic–thermal synergistic liquid egg sterilization system is shown in Figure 1.

2.2. Design of Ultrasonic Sterilization System Device

The ultrasonic generator system consists of two ultrasonic generators, each connected to two sets of ultrasonic transducers. One generator is a 20 kHz single-frequency, controlling two transducers with frequencies both set at 20 kHz. The other generator is a switchable dual-frequency generator with 28 kHz and 40 kHz, connected to two dual-frequency

transducers with frequencies of 28 kHz and 40 kHz, respectively. The driving power of the 20 kHz ultrasonic generator is 600 W, with a maximum current of 5 A. The driving power of the 28/40 kHz ultrasonic generator is 300 W, with a maximum current of 4.5 A.

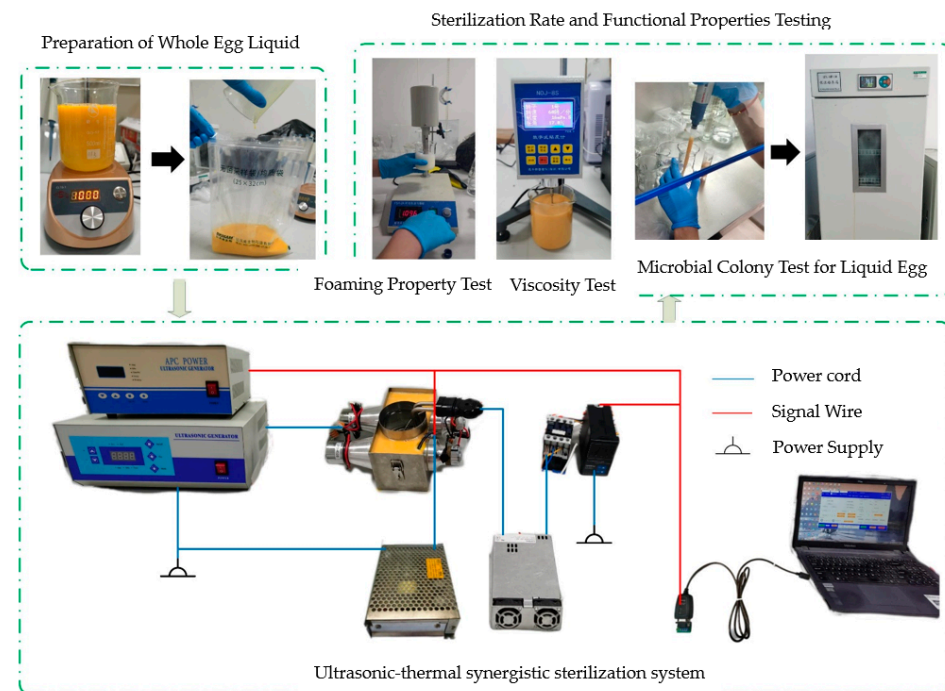


Figure 1. Ultrasonic–thermal synergistic sterilization system proposal for liquid eggs.

The ultrasonic generator mainly consists of a rectification and filtering circuit, a BUCK chopping circuit, a high-frequency inverter circuit, and a transformer. Its operation process is as follows: when connected to 220 V AC power, the rectification and filtering circuit first convert the AC voltage into 315 V DC voltage. Subsequently, to adjust the output power, the DC chopping circuit outputs DC voltage. To obtain smooth DC, a low-pass filter is used to adjust the BUCK chopping circuit. Simultaneously, a large capacitor filtering capacitor is used to obtain low-ripple DC. Adjust the DC current to the desired voltage, and, finally, through the high-frequency inverter circuit and transformer, convert the DC current into the high-frequency electrical signal required by the ultrasonic transducer, thereby causing the ultrasonic transducer to vibrate and operate. The framework diagram and physical diagram of the ultrasonic generator system are shown in Figures 2 and 3, respectively.

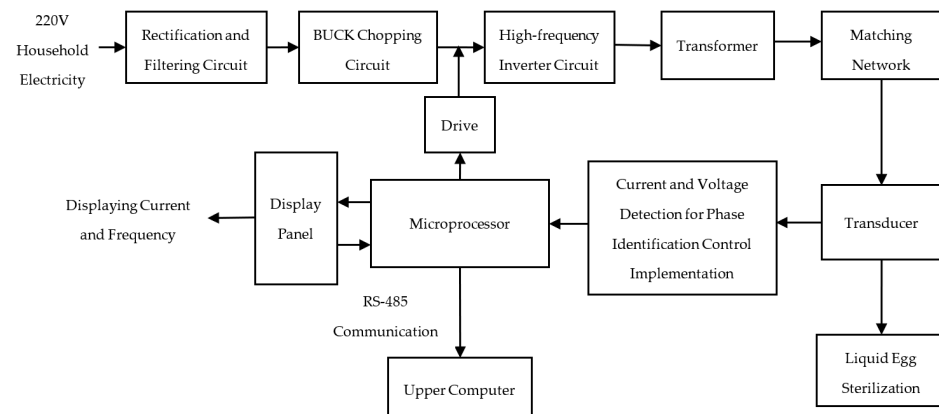


Figure 2. Schematic diagram of the ultrasonic generator system.

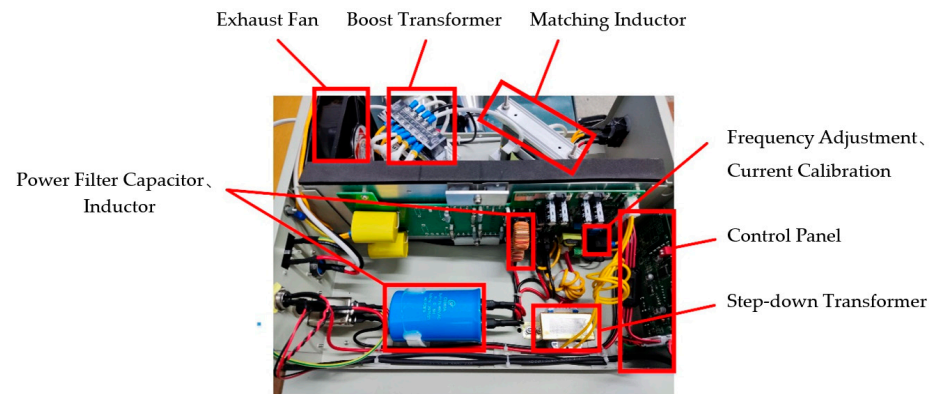


Figure 3. Physical diagram of the ultrasonic generator circuit.

2.3. Design of Constant Temperature Water Bath System

The constant temperature water bath provides a stable heat source for the heating of liquid eggs and serves as an additional sterilization effect based on temperature. The temperature control part of the constant temperature heating device uses a temperature sensor inside the water bath for temperature feedback control. The heating part of the device connects the low-voltage output of the constant temperature controller to the AC contactor to control the starting and stopping of the 24 V power supply, indirectly controlling the operation of the heater to ensure temperature stability. Different temperatures can be set to synergize with the ultrasonic generator for sterilization experiments.

The constant temperature water bath system is primarily controlled by the constant temperature controller. The upper computer can set different temperatures and action times based on the ultrasonic frequency, thus working together for synergistic sterilization. The main hardware includes a constant temperature controller supporting RS485 communication, a 24 V power supply, an AC contactor, a heater, and a temperature sensor. The overall hardware structure diagram of the constant temperature water bath system is shown in Figure 4.

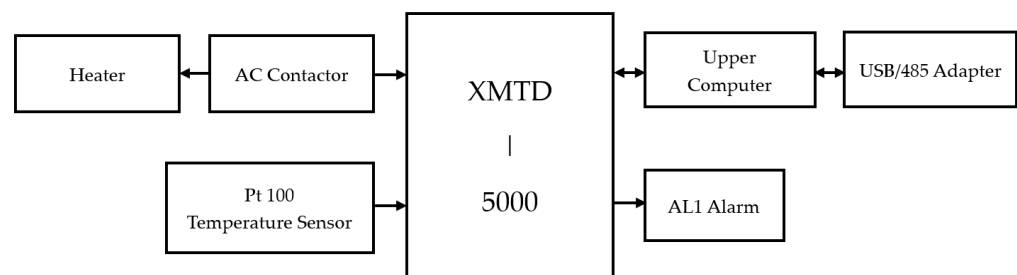


Figure 4. Overall hardware structure diagram of the constant temperature water bath system.

2.4. Control Software Design

The ultrasonic–thermal synergistic sterilization control system adopts the form of “PC + Modbus-RTU communication protocol”. Through software developed on the PC, it communicates via the RS485 bus with devices such as ultrasonic generators, temperature sensors, and constant temperature controllers in the lower machine that have Modbus communication protocol. It sends read and write commands to the microcontroller registers and coils of each device, controlling the collaborative work of devices and data collection.

The functions of the ultrasonic–thermal synergistic control software are mainly managed by three modules: (1) function selection module, (2) ultrasonic water bath synergistic control module, and (3) intelligent processing module. The upper computer software communicates via the ModbusRTU protocol with the ultrasonic generator and the constant temperature controller, and, after receiving signals in the microcontroller registers, the temperature controller heats the water bath to the specified temperature. Simultaneously, it

changes the start–stop status and frequency of the two ultrasonic generators to achieve the effects of multiple frequencies in synergy with water bath constant temperature heating for sterilization. After reaching the designated time point, the ultrasonic generator and constant temperature controller will stop working. The PC simultaneously reads data, such as the actual working frequency of the ultrasonic transducer, the sterilization temperature, and other status information, and saves it.

2.5. Simulation of Ultrasonic–Thermal Synergistic Action Based on COMSOL

In order to achieve multiphysics simulation of multi-frequency ultrasonic transducers and guide the design of transducer layout, simulations of both a single ultrasonic transducer and a multi-frequency ultrasonic transducer were conducted using the “Acoustic–Piezoelectric Interaction, Time Domain” analysis module in the COMSOL 6.0 software.

2.5.1. Parameter Settings

The effective range covered by the ultrasonic transducer is approximately 100 mm along the acoustic axis, with coverage on both sides of the acoustic axis being approximately 50 mm each. The sterilization container can be designed as a rectangular container with dimensions of 200 mm × 100 mm × 100 mm to ensure that the ultrasonic waves cover the entire container. The simulated ultrasonic frequency is 28 kHz, and the mesh size in the liquid domain is set between 2.68 mm and 5.36 mm. The meshing of the ultrasonic transducer section is chosen to be automatically partitioned by the system, as shown in Figure 5. The main simulation parameters are presented in Table 1.

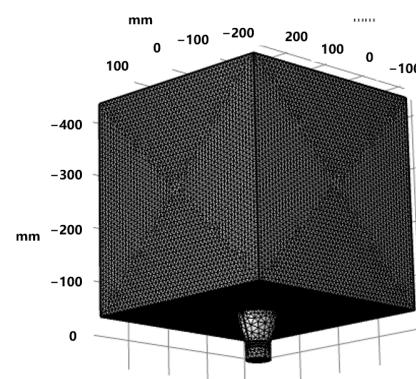


Figure 5. Simulation grid division.

Table 1. Main simulation parameters in COMSOL.

Name	Project	Parameter
Ultrasonic Transducer	Piezoelectric Material	PZT-4
	Back Cover	45 Steel
	Front Cover	YL-12 Hard Aluminum
	Sinusoidal Excitation Voltage	336 V
	Grid Division	Standard Size
Acoustic Propagation Domain	Liquid Egg Density	997 kg/m ³
	Speed of Sound in Egg Liquid	1500 m/s
	Grid Division	2.68–5.36 mm
	Liquid Egg Temperature	50 °C

Multi-frequency transducers with frequencies of 20 kHz and 40 kHz are arranged with four ultrasonic transducers distributed along the two long sides of the container. The center distance between transducers is 100 mm. In the boundary settings, the middle surface of the piezoelectric ceramic of the four ultrasonic transducers is set as the positive pole, while the other eight surfaces are set as the negative pole. The ultrasonic frequencies at the

voltage boundaries of the piezoelectric material are set to 20 kHz and 40 kHz, with voltages being sinusoidal wave excitations measured in practice. According to the multi-frequency ultrasonic frequencies, the mesh size is divided into one-fifth to one-tenth of the wavelength. The meshing is shown in Figure 6, and the main simulation parameters are set as shown in Table 2.

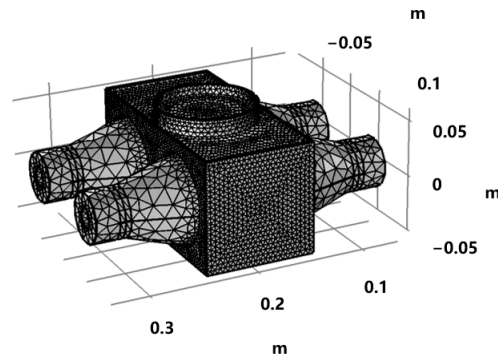


Figure 6. Mesh division for multi-frequency simulation.

Table 2. Main parameter settings for multi-frequency ultrasonic simulation.

Name	Project	Parameter
Ultrasonic Transducer	Piezoelectric Material	PZT-4
	Grid Division	Standard Size
	Container Material	304 Stainless Steel
	Liquid Egg Density	997 kg/m ³
Liquid Egg Sterilization Area	Grid Division	3.75–7.5 mm
	Liquid Egg Temperature	50 °C

2.5.2. Simulation Results

The simulated sound pressure and propagation attenuation curve of a single ultrasonic transducer under excitation are shown in Figure 7a,b. The wave-radiating sound field of a single ultrasonic transducer exhibits clear directionality, with energy concentrated mainly near the center axis of the transducer. The amplitude of the sound pressure along the acoustic axis shows a rapid exponential decay, attenuating to around Pa at a distance of $z = 100$ mm from the sound source, and gradually leveling off thereafter. Therefore, the effective range covered by a single ultrasonic transducer can be approximately 100 mm along the acoustic axis, with coverage on both sides of the acoustic axis being approximately 50 mm.

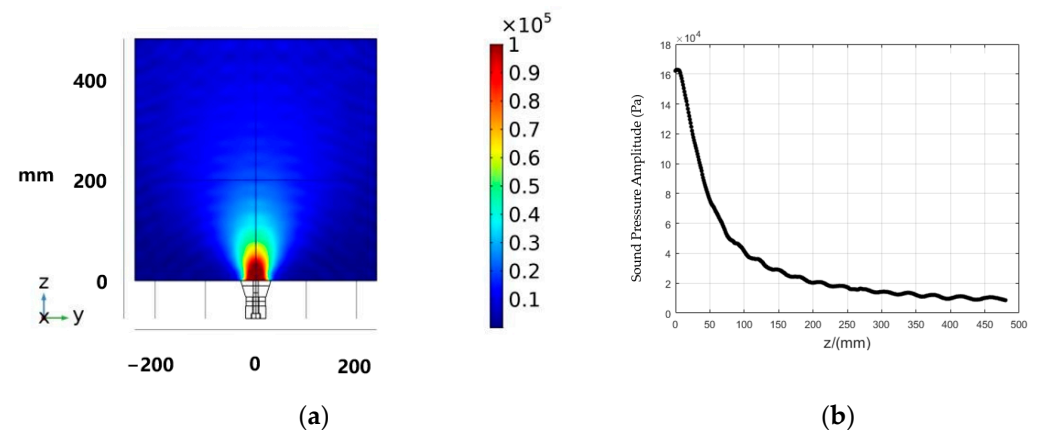


Figure 7. Simulation diagram of a single transducer: (a) single oscillator simulation diagram; (b) change in pressure amplitude along the Z direction.

The distribution of the sound field and the sound pressure curve of the multi-frequency ultrasonic array are shown in Figure 8a,b. The arrangement of transducers in the multi-frequency ultrasonic array can increase the acoustic intensity in the ultrasonic field. After the stability of the ultrasonic field is achieved, the sound pressure stabilizes at Pa. The designed multi-frequency transducer scheme creates a reverberant sound field with two different frequencies of ultrasonic waves in the enclosed space, enhancing and evenly distributing the cavitation effect, ensuring uniform acoustic intensity coverage throughout the entire container.

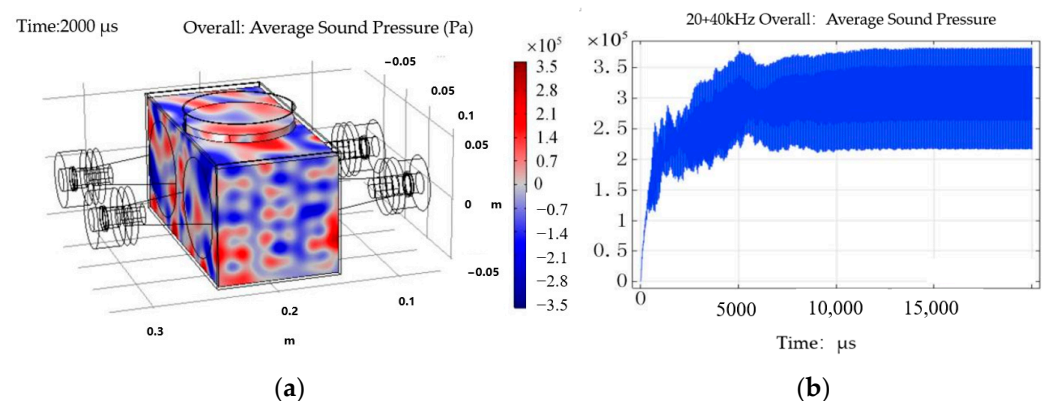


Figure 8. Simulation diagram of sound field distribution for multi-frequency transducer ultrasonics: (a) simulation diagram of 20 + 40 kHz; (b) changes in maximum total sound pressure in the body.

2.6. Verification of Software Functions for Ultrasonic–Thermal Synergistic Sterilization System

To validate the data acquisition and processing functions of the ultrasonic–thermal synergistic control software, as well as the accuracy of multi-frequency ultrasonic field distribution and pressure simulation, experiments were conducted under the operation of the ultrasonic–thermal synergistic control software to collect and process data on the propagation of multi-frequency ultrasonic waves in liquid egg medium. The software was set to 20 + 40 kHz, and the constant temperature heating temperature was set to 50 °C. The collected data included the current of ultrasonic transducers 1 and 2, the sweep frequency of the ultrasonic generator, the communication rate between all instruments and the upper computer, the real-time sterilization temperature of the liquid eggs, and the real-time sterilization time (countdown according to the designed time). The experimental data collection and processing document are shown in Figure 9.

	Current of Machine 1	Frequency of Unit 1	Communication of Unit 1	Current of Machine 2	Frequency of Unit 2	Communication of Unit 2	Sterilization temperature	Sterilization time
1	0.6	39.8	9600	0.5	19.8	9600	50.0	20
2	0.5	39.9	9600	0.6	19.7	9600	49.8	19
3	0.5	39.8	9600	0.6	19.9	9600	49.7	18
4	0.5	39.8	9600	0.6	19.9	9600	49.7	17
5	0.6	40.1	9600	0.6	20.0	9600	49.9	16
6	0.6	40.0	9600	0.6	20.0	9600	50.1	15
7	0.6	39.9	9600	0.5	20.0	9600	50.0	14
8	0.6	40.0	9600	0.6	19.9	9600	49.9	13
9	0.6	39.9	9600	0.6	19.9	9600	49.8	12
10	0.6	39.9	9600	0.6	19.9	9600	49.7	11
11	0.6	39.9	9600	0.5	19.8	9600	49.7	10
12	0.6	39.9	9600	0.6	20.0	9600	49.6	9
13	0.5	39.9	9600	0.6	20.0	9600	49.8	8
14	0.6	39.9	9600	0.5	20.0	9600	49.9	7
15	0.6	39.9	9600	0.6	20.0	9600	50.0	6
16	0.5	40.0	9600	0.5	19.8	9600	50.1	5
17	0.6	39.8	9600	0.6	19.9	9600	50.2	4
18	0.5	39.9	9600	0.6	19.8	9600	50.0	3
19	0.6	40.0	9600	0.6	19.8	9600	50.0	2
20	0.6	39.8	9600	0.6	19.7	9600	49.9	1

Figure 9. Data collection and processing chart.

As shown in Figure 9, it is evident that the ultrasonic frequency remains stable, with fluctuations within 0.3 kHz of the set frequency. The current in the ultrasonic generator is stable in the optimal working state of the transducer, and the liquid egg temperature is stable with fluctuations within 0.3 °C of the set temperature. Therefore, it can be concluded that the ultrasonic–thermal synergistic sterilization software played a role in controlling the start and stop of the ultrasonic and constant temperature devices and in monitoring the stability of the ultrasonic–thermal synergistic action during the experiment.

To validate whether the effect of the ultrasound–thermal synergistic device corresponds to the simulation results, an acoustic intensity test of liquid eggs under the operation of the ultrasound–thermal synergistic sterilization system was conducted. The experimental setup is shown in Figure 10a. The model of the ultrasonic acoustic intensity detection device is YP0511F. The measured duration of acoustic intensity is 20 s, with a value recorded every 1 s. The simulated volumetric average acoustic intensity ranges from 1.05 to 1.5 W/cm², which is in good agreement with the actually measured field intensity. After 20 measurements, the average acoustic intensity can reach 1.24 W/cm², achieving a certain sterilization effect, as shown in Figure 10b.

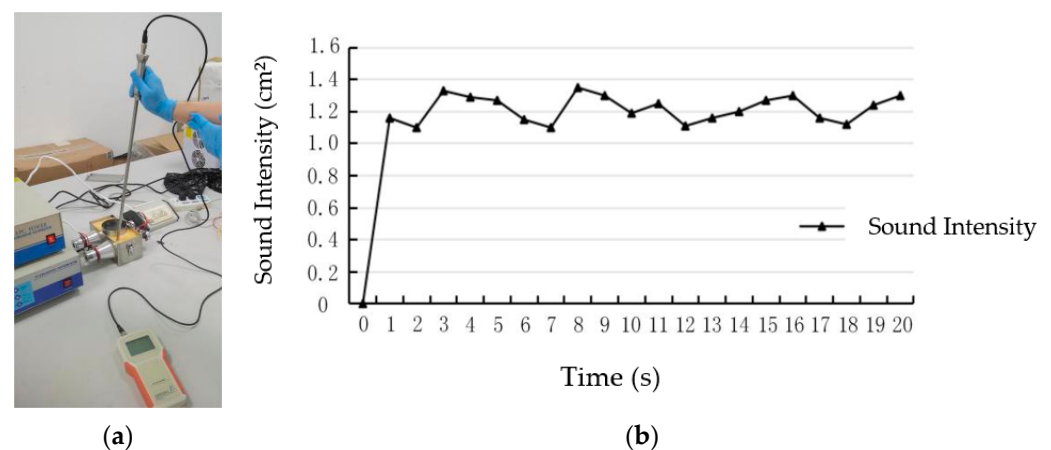


Figure 10. Ultrasonic measuring instrument and measured data diagram: (a) ultrasonic acoustic intensity measurement device; (b) measured acoustic intensity data.

This section established a simulation model of an ultrasound–thermal synergistic sterilization system and validated the sterilization simulation model under the action of multi-frequency ultrasound using COMSOL 6.0 software. The results indicate that, after the pressure stabilizes within the multi-frequency ultrasound sterilization simulation model, ultrasound waves can cover the entire sterilization container, and the intensity of the average acoustic pressure within the model can meet the sterilization requirements. A synergistic ultrasound–thermal liquid egg sterilization test platform was constructed according to the design requirements, and a detailed introduction to the hardware functions and parameters was provided. An ultrasound–thermal synergistic sterilization software control system based on the Modbus communication protocol was established. The effectiveness of the ultrasound–thermal synergistic sterilization control software, and the data acquisition and processing functions, were verified through experiments, and the simulation was instrumentally validated to be in agreement with actual measured acoustic intensities, thereby confirming the accuracy of the sterilization simulation model.

2.7. Materials and Equipment for Ultrasonic–Thermal Synergistic Sterilization Experiment

Fresh eggs were purchased from the local fresh produce market; nutrient agar medium was obtained from Beijing ComWin Biotech (China) Co., Ltd.; chemicals such as sodium chloride and distilled water were procured from China National Pharmaceutical Group Corporation; and all reagents were of analytical grade (AR). Sterile operating table (SW-CJ-2D), Jinan Senya Experimental Instrument (China) Co., Ltd.; vertical pressure steam sterilizer

(LX-B50L), Hefei Huatai Medical Equipment (China) Co., Ltd.; adjustable homogenizer (FSH-2A), Changzhou Tianrui Instrument (China) Co., Ltd.; small magnetic stirrer (CL19-1), Shanghai Sile Instrument (China) Co., Ltd.; electronic balance (LE204E/02), precision pH meter (S400-K), Mettler Toledo Technology (China) Co., Ltd.; constant temperature incubator (ADX-SHP-160), Wuhan Andexin Detection Equipment (China) Co., Ltd.

2.8. Experimental Design

2.8.1. Experimental Procedure

(1) Preparation of liquid whole egg: wash the eggs with warm water, sterilize with alcohol, crush under aseptic conditions, filter out the chalaza and egg yolk membrane, stir evenly with a magnetic stirrer at a speed of 1000 r/min, and store the liquid eggs in a sterile sealed bag in a refrigerator at 4 °C; (2) single-factor experiment: referring to the sterilization levels of Lee [21], this experiment controls the effects of three factors: temperature (50, 55, 60 °C), time (7, 11, 15 min), and frequency (20, 20 + 28, 20 + 40 kHz) on the sterilization rate of liquid eggs; (3) response surface analysis experiment: based on the results of single-factor experiments, with temperature, time, and frequency as variables, a Box–Behnken design was used to conduct a 3-factor 3-level experiment to optimize the sterilization conditions. The optimization process of ultrasound-assisted thermal sterilization parameters is shown in Figure 11.

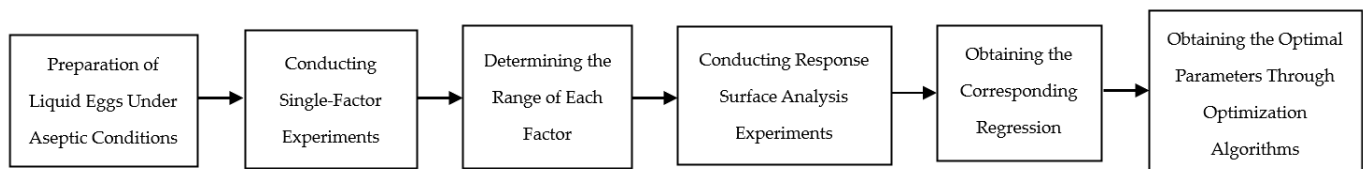


Figure 11. Ultrasonic collaborative thermal sterilization process.

2.8.2. Microbial Quantification

According to GB/T 4789.2-2016, colony counts are performed on liquid egg white samples before and after sterilization treatment. Colony counts are expressed in colony-forming units (CFU). The sterilization effect is indicated by the lethal rate, calculated using the following formula:

$$\text{Lethal logarithm} = \lg(N_0/N) \quad (1)$$

N_0 : Microbial count before sterilization treatment, CFU/mL; N : Microbial count after sterilization treatment, CFU/mL.

2.8.3. Determination of Sterilization Rate of Liquid Eggs

The determination of total bacterial count refers to GB/T 4789.2-2016 “Microbiological examination of food hygiene—Part 2: Examination of total number of colonies.” According to the national standard, the colony counts of the control group and experimental group are obtained, and their sterilization rates are calculated.

The bacterial lethality is calculated using the following formula:

$$\text{Bacterial lethality}/\% = \frac{D - E}{D} \times 100 \quad (2)$$

In the formula, D represents the bacterial count of the sample without ultrasonic treatment, and E represents the bacterial count of the sample after ultrasonic treatment.

2.8.4. Determination of Physicochemical Properties of Liquid Eggs

(1) Determination of Foaming Capacity and Foaming Stability

Liquid eggs, with their excellent foaming properties, are widely used in the baking of products such as cakes and desserts. The study of the foaming capacity and foaming stability functional properties of liquid eggs is particularly important. After ultrasonic

treatment, the homogeneous liquid sample (5 mL) is placed in a small test tube, and distilled water is added to make a 5% dilution solution with a volume of 100 mL. The liquid volume is recorded at this time. Under the action of a stirrer at 10,000 r/min, it is stirred for 1 min. The foaming volume and liquid volume are analyzed and recorded just after stirring stops and 30 min after stirring, and the foaming capacity (FC) and foaming stability (FS) are determined.

Foaming capacity (FC) is calculated using the following formula:

$$FC = (V_1 - V_0) / V_0 \times 100\% \quad (3)$$

Foaming stability (FS) is determined using the following formula:

$$FS = (V_2 - V_0) / (V_1 - V_0) \times 100\% \quad (4)$$

where: V_1 is the foam volume of the sample liquid at zero time after stirring (mL) and V_2 is the volume after standing for 30 min.

(2) Determination of pH Value

The sample is placed in a beaker, and the pH is measured at room temperature using a pH meter.

(3) Determination of Viscosity

Modified from the method in reference [23]. Take 30 mL of the sample in a 50 mL beaker and measure the viscosity at room temperature using a rotational viscometer. Testing conditions: select rotor 1 and measure the viscosity of the liquid whole egg sample at 60 rpm.

3. Results

3.1. Single-Factor Test Results

Single-factor changes in ultrasound frequency, temperature, and exposure time were analyzed for their impact on sterilization efficiency and foaming properties. The experimental results are shown in Figure 12. First, the heat treatment temperature was set from 40 °C to 60 °C, with a treatment time of 11 min and an ultrasound frequency of 20 + 40 kHz, analyzing the impact of temperature changes in synergistic sterilization experiments, as shown in Figure 12a. From Figure 12a, it can be observed that as the temperature gradually increases, the sterilization effect improves. However, when the temperature exceeds 55 °C, a critical property for liquid eggs, foaming ability, begins to significantly decline. This indicates that at this point, the temperature starts to have a negative impact on the foaming ability of liquid eggs. The effects of different treatment times on the sterilization rate and foaming properties were investigated at a treatment temperature of 50 °C, treatment frequency of 20 + 40 kHz, and treatment times of 0, 3, 6, 9, 12, and 15 min, as shown in Figure 12b. From Figure 12b, it can be observed that as the treatment time increases, the sterilization rate generally shows an upward trend. However, with the prolongation of time, the critical physicochemical property of foaming ability for liquid eggs exhibits a significant decline after 12 min of operation in the sterilization system, indicating that the temperature at this point begins to have a negative impact on the foaming ability of liquid eggs. The influence of different frequencies on the sterilization rate and foaming properties was examined with a treatment time of 11 min, treatment temperature of room temperature (25 °C), and treatment frequencies of 20 + 28 kHz, 20 + 40 kHz, 20 kHz, 28 kHz, and 40 kHz, as shown in Figure 12c. From Figure 12c, it can be seen that the multi-frequency combination sterilization of 20 + 28 kHz and 20 + 40 kHz achieves a higher sterilization rate compared to single-frequency sterilization at 20 kHz, 28 kHz, and 40 kHz. The foaming ability of liquid eggs shows no significant fluctuations. The ultrasonic frequencies have a greater impact on the sterilization rate of liquid eggs and a lesser impact on their foaming ability.

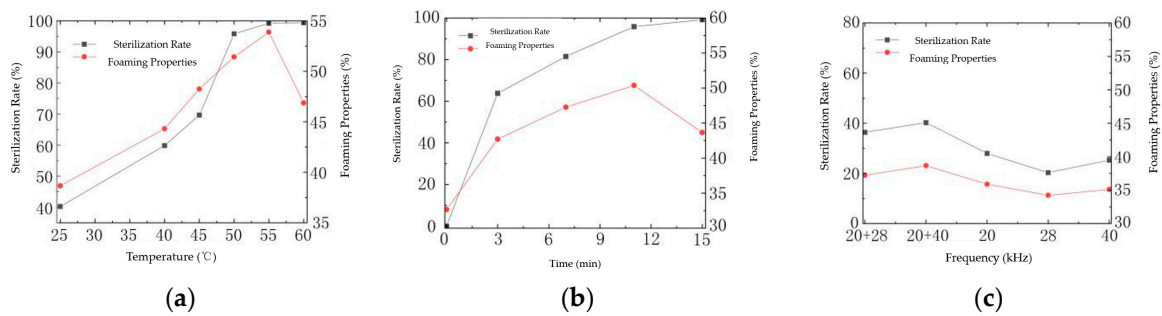


Figure 12. Single factor experimental data analysis diagrams: (a) the effect of temperature on sterilization rate and foaming properties of liquid eggs; (b) the effect of time on sterilization rate and foaming properties of liquid eggs; (c) the effect of frequency on sterilization rate and foaming properties of liquid eggs.

The sterilization test colony images are shown in Figure 13. The purpose of Figure 13 is to observe the single-factor effects of temperature, time, and frequency on the sterilization rate, decimal reduction time, and foaming properties of the target under the ultrasound–thermal synergistic sterilization control system through experiments. Figure 13a represents the colony image of the untreated experiment. In Figure 13b, based on the results of Figure 12a, a treatment time of 11 min and ultrasound frequencies of 20 + 40 kHz were selected. Under a temperature of 60 °C, the sterilization rate increased from 41.25% to 99.93%. The calculation of the decimal reduction time showed an increase of 2.92 log values as the sterilization temperature increased from 25 °C to 60 °C. In Figure 13c, based on the results of Figure 12b, a treatment temperature of 50 °C and frequencies of 20 + 40 kHz for a treatment time of 15 min resulted in a final sterilization rate of 99.95%. The calculation of the decimal reduction time showed an increase of 3.30 log values as the treatment time increased from 0 to 15 min. In Figure 13d, based on the results of Figure 12c, a treatment time of 11 min at room temperature (25 °C) with a frequency of 20 + 40 kHz achieved a highest sterilization rate of 39.95%, with a decimal reduction time increase of 0.22 log values. It was concluded that the sterilization temperature and treatment time had a significant impact on the sterilization rate and decimal reduction time, while the choice of ultrasound frequency had a smaller effect on these parameters. However, the ultrasound frequency had a minor impact on the foaming properties of liquid eggs. Therefore, considering both the sterilization rate and foaming properties, a temperature range of 50 °C to 60 °C, treatment time of 7 to 15 min, and an ultrasound frequency of 20 + 40 kHz were selected for subsequent ultrasound–thermal synergistic sterilization tests.

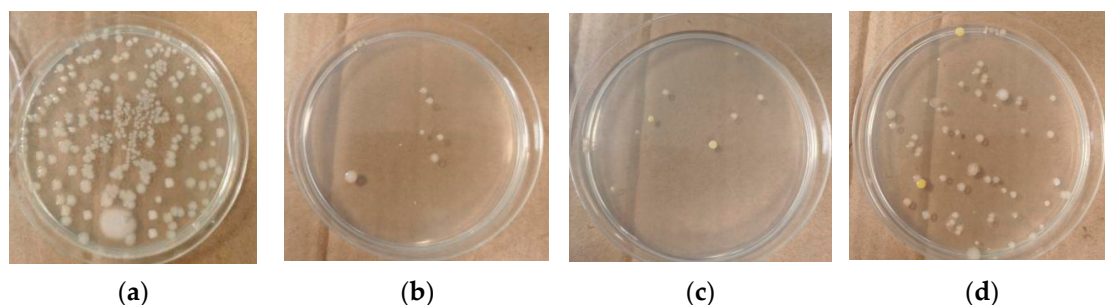


Figure 13. Test verification part comparison diagrams: (a) the colony image of the untreated experiment (diluted 100 times); (b) the colony image of the sterilization experiment with a treatment time of 11 min, an ultrasonic frequency of 20 + 40 kHz, and a temperature of 60 °C (diluted 10 times); (c) the colony image of the sterilization experiment with a treatment temperature of 50 °C, an ultrasonic frequency of 20 + 40 kHz, and a treatment time of 15 min (diluted 10 times); (d) the colony image of the sterilization experiment with a treatment time of 11 min, a treatment temperature of 25 °C, and an ultrasonic frequency of 20 + 40 kHz (diluted 100 times).

3.2. The Impact of Ultrasound–Thermal Synergistic Sterilization System on the Functional Properties of Liquid Eggs

3.2.1. Foaming Capacity and Foaming Stability

Foaming capacity is one of the key quality characteristics that egg processing focuses on, mainly influenced by factors such as the composition and structure of egg white, environmental conditions, and production and processing technology [7]. The impact of the ultrasound–thermal synergistic sterilization system on the foaming properties of egg liquid is shown in Figure 14.

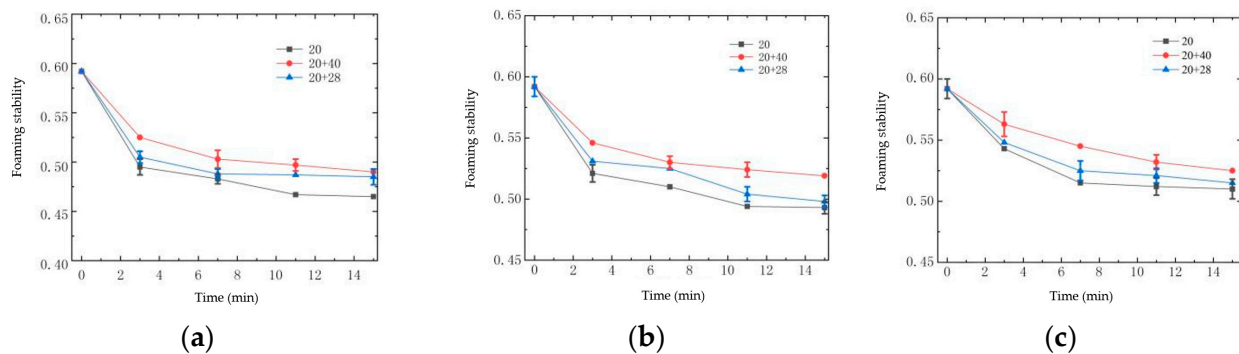


Figure 14. Influence of ultrasonic combined with heat on the stability of liquid egg foaming: (a) influence of synergistic ultrasound at 50 °C on foaming stability; (b) influence of synergistic ultrasound at 55 °C on foaming stability; (c) influence of synergistic ultrasound at 60 °C on foaming stability.

Figure 14a–c display the curves of the changes in foaming properties of egg liquid under the influence of temperature, time, and frequency, respectively. Under the action of different ultrasound frequencies at the same temperature, the 20 + 40 kHz multifrequency ultrasound shows the best foaming effect. Under the synergistic action of ultrasound at different temperatures, with the increase of ultrasound treatment time, the foaming properties of egg liquid first increase and then decrease, reaching optimal foaming properties between 55 °C and 60 °C. Under the synergistic action of ultrasound at the same frequency and the same temperature, foaming properties show an initial increase followed by a decrease, and when the ultrasound–thermal synergistic action exceeds 12 min, foaming stability will rapidly decrease.

Foaming stability is a measure of the persistence of foam formation, often determined by factors such as liquid discharge from the foam film, membrane rupture, and disproportionation. Using untreated blank egg white as the control group, the changes in foaming stability of egg liquid under different process parameters are shown in Figure 14.

Processed with simultaneous ultrasound treatment at 50 °C, 55 °C, and 60 °C, respectively, Figure 14a–c show the curves of the change in foaming stability of liquid eggs with different ultrasound frequencies (20 kHz, 20 + 40 kHz, and 20 + 28 kHz) over time. The multi-frequency ultrasound at 20 + 40 kHz exhibits the best effect on foaming stability. Under the same temperature coordination, with the increase in ultrasound exposure time, the foaming stability of liquid eggs generally shows a decreasing trend followed by stabilization. As the temperature rises from 50 °C to 60 °C, foaming stability gradually increases, and the influence of temperature and time on foaming stability is relatively stable.

3.2.2. Viscosity

Liquid eggs are widely used in food production and viscosity is an important property influencing their application. Using untreated blank eggs as a control group, the viscosity changes of liquid eggs under different process parameters were obtained. The results are shown in Figure 15.

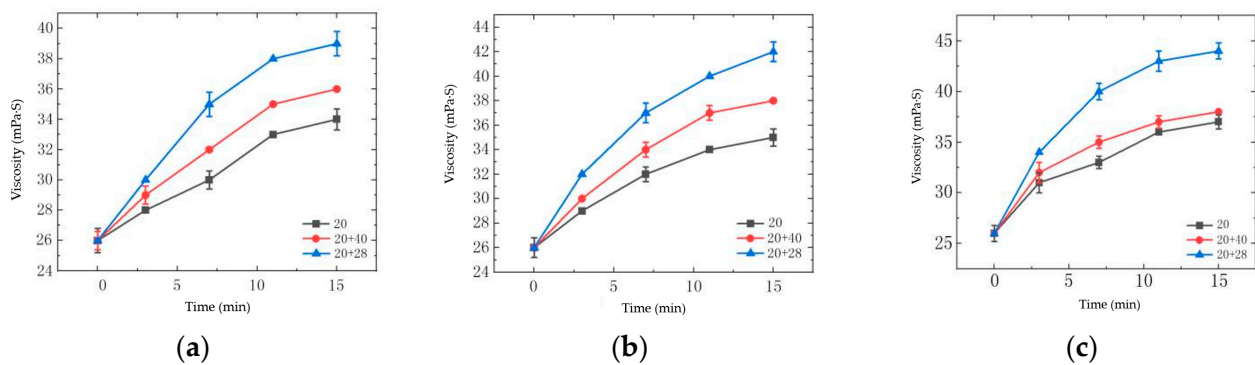


Figure 15. Effect of ultrasonic combined with heat on viscosity of liquid eggs: (a) effect of synergistic ultrasound at 50 °C on viscosity; (b) effect of synergistic ultrasound at 55 °C on viscosity; (c) effect of synergistic ultrasound at 60 °C on viscosity.

Subjected to synergistic ultrasound treatment at 50 °C, 55 °C, and 60 °C, respectively, Figure 15a–c show the viscosity changes of liquid eggs under the influence of different ultrasound frequencies (20 kHz, 20 + 40 kHz, and 20 + 28 kHz) over time. The multi-frequency ultrasound at 20 + 28 kHz has the greatest impact on viscosity. Under the synergistic action at the same temperature, the viscosity of liquid eggs generally shows an increasing trend with the increase of ultrasound treatment time. As the temperature rises from 50 °C to 60 °C, the viscosity gradually increases.

3.2.3. pH

Protein is the most abundant nutrient in liquid eggs and the pH value shows a significant positive correlation with the solubility of proteins [24]. Therefore, the change in pH can be used to assess the variation in protein solubility in liquid eggs. The comparative results are shown in Figure 16.

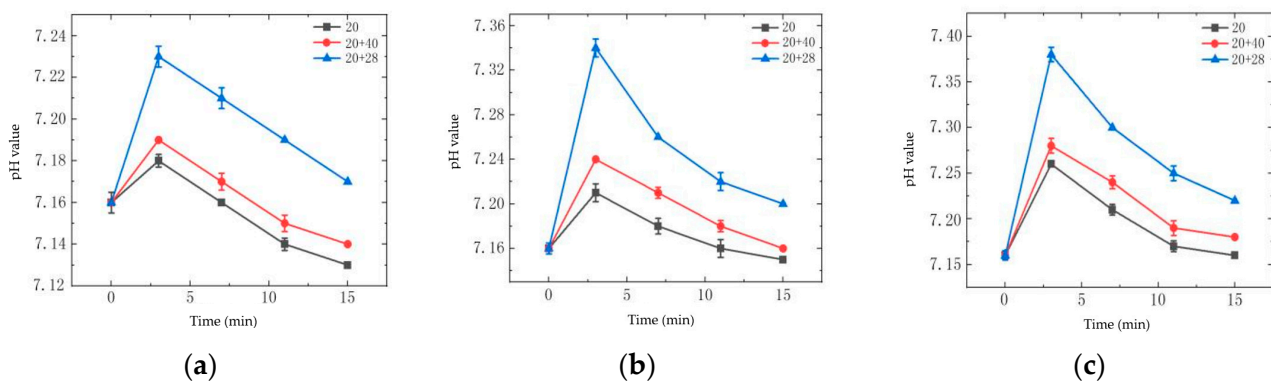


Figure 16. Effect of ultrasonic combined with heat on pH value of liquid eggs: (a) influence of synergistic ultrasound at 50 °C on pH values; (b) influence of synergistic ultrasound at 55 °C on pH values; (c) influence of synergistic ultrasound at 60 °C on pH values.

The pH values of liquid eggs under synergistic ultrasonic treatment at 50 °C, 55 °C, and 60 °C, respectively, are shown in Figure 16a–c for different ultrasonic frequencies (20 kHz, 20 + 40 kHz, and 20 + 28 kHz) over time. The multi-frequency ultrasound at 20 + 28 kHz has the greatest impact on pH values. Under the synergistic action at the same temperature, the pH values of liquid eggs generally increase first and then decrease with the increase in ultrasonic treatment time. As the temperature rises from 50 °C to 60 °C, the pH values gradually increase. However, the overall change in pH is not significant and remains within a reasonable range.

3.3. Response Surface Experiment of Ultrasound–Thermal Synergistic Sterilization

3.3.1. Response Surface Experiment Design

According to the principles of the response surface Box–Behnken design, and combined with the results of single-factor experiments, a response surface analysis experiment with three factors and three levels, including sterilization temperature, processing time, and ultrasonic frequency, was designed. The analysis was conducted using sterilization rate as the response variable, and a regression equation and model were obtained. The optimal parameters for the ultrasonic–thermal synergy on liquid egg sterilization were determined using the regression equation and model through an algorithm, as shown in Table 3.

Table 3. Response surface experiment design table.

Level	Factors		
	A Temperature (°C)	B Processing Time (min)	C Ultrasonic Frequency (kHz)
−1	50	7	20 + 28
0	55	11	20 + 40
1	60	15	20

3.3.2. Establishment of Regression Models and Significance Analysis

Based on single-factor experiments, a response surface Box–Behnken design was employed by selecting three factors: temperature (A), processing time (B), and ultrasonic frequency (C) to investigate their effects on sterilization rate (Y_1) and foaming properties (Y_2). The experimental designs for each group and the sterilization rate results are presented in Table 4. Design-Expert.V8.0.6.1 software was employed for multivariate regression fitting between the designs, resulting in quadratic regression equations with sterilization rate (Y_1) and foaming properties (Y_2) as the respective target functions:

$$\begin{aligned} Y_1 &= -296.5721 + 10.6850A + 13.1559B - 15.1431C - 0.1694AB + 0.2535AC + 0.001875BC - 0.07219A^2 - 0.1325B^2 - 3.0448C^2 \\ Y_2 &= -766.5755 + 29.0644A + 6.27175B - 0.0150C - 0.1375AB - 0.001875AC - 0.2673BC - 0.24478A^2 - 0.24478B^2 - 2.7990C^2 \end{aligned} \quad (5)$$

According to the analysis of variance in Tables 5 and 6, both experimental design models are highly significant with $p < 0.01$; lack-of-fit terms with $p > 0.05$ are not significant, indicating that the established quadratic regression model can effectively predict the response values. The significance analysis results of the regression model coefficients in Tables 5 and 6 reveal that, under the selected single-factor conditions, the factors affecting the sterilization rate (Y_1) are arranged in descending order of importance: temperature (A) > processing time (B) > ultrasonic frequency (C); A, B, AB, C^2 have a highly significant impact on the indicator ($p < 0.01$); C, A^2 , B^2 have a significant impact on the indicator ($p < 0.05$), and the impact of other factors is not significant. The factors influencing the sterilization rate (Y_2) are arranged in descending order of importance: temperature (A) > ultrasonic frequency (C) > processing time (B). A^2 has a highly significant impact on the indicator ($p < 0.01$), A, B^2 has a significant impact on the indicator, and the impact of other factors is not significant.

Table 4. Experiment design and results for response surface analysis.

Experiment ID	A	B	C	Y_1 Sterilization Rate (%)	Y_2 Foaming Properties (%)
1	−1	−1	0	93.46	47.39
2	1	−1	0	97.56	43.35
3	−1	1	0	99.25	43.65
4	1	1	0	99.80	38.41
5	−1	0	−1	93.57	48.48

Table 4. Cont.

Experiment ID	A	B	C	Y ₁ Sterilization Rate (%)	Y ₂ Foaming Properties (%)
6	1	0	−1	99.33	43.65
7	−1	0	1	96.32	45.70
8	1	0	1	99.15	39.64
9	0	−1	−1	91.89	46.56
10	0	1	−1	97.67	51.90
11	0	−1	1	94.87	42.28
12	0	1	1	95.68	47.59
13	0	0	0	99.32	53.90
14	0	0	0	98.41	52.27
15	0	0	0	99.61	51.82
16	0	0	0	99.45	55.64
17	0	0	0	97.92	55.36

Table 5. Variance analysis of bactericidal rate regression model.

	Sum of Squares	Degrees of Freedom	Mean Square	F-Value	p-Value
Model	407.88	9	45.32	26.39	0.0001
A	155.23	1	155.23	90.39	<0.0001
B	109.67	1	109.67	63.86	<0.0001
C	11.14	1	11.14	6.49	0.0383
AB	45.90	1	45.90	26.73	0.0013
AC	6.43	1	6.43	3.74	0.0943
BC	0.00025	1	0.00025	0.000131	0.9912
A ²	13.71	1	13.71	7.99	0.0256
B ²	18.92	1	18.92	11.02	0.0128
C ²	39.03	1	39.03	22.73	0.0020
Residual Term	12.02	7	1.72		
Lack-of-fit Term	9.85	3	3.28	6.04	0.0575
Pure Error	2.17	4	0.54		
R ²	0.9714				
CV/%	1.37				

Table 6. Analysis of variance of bubbling regression model.

	Sum of Squares	Degrees of Freedom	Mean Square	F-Value	p-Value
Model	394.80	9	43.87	5.17	0.0207
A	49.85	1	49.85	5.88	0.0458
B	0.4851	1	0.49	0.0572	0.8178
C	30.34	1	30.34	3.58	0.1005
AB	0.36	1	0.36	0.0424	0.8426
AC	0.27	1	0.27	0.0313	0.8426

Table 6. Cont.

	Sum of Squares	Degrees of Freedom	Mean Square	F-Value	p-Value
BC	0.0002	1	0.0002	0.0001	0.9960
A ²	187.97	1	187.97	22.16	0.0022
B ²	64.59	1	64.59	7.62	0.0281
C ²	32.99	1	32.99	3.89	0.0892
Lack-of-fit Term	47.27	3	15.76	5.21	0.0723
Pure Error	12.09	4	3.02		
R ²	0.9032				
CV/%	6.13				

3.3.3. Interaction Effects in Response Surface Models

The response surface plots of the model are shown in Figures 17–19. From Figure 17, it can be observed that the surface slope of sterilization temperature is larger than that of processing time, indicating a more significant impact of sterilization temperature on sterilization rate. Figure 18 illustrates that during the transformation of ultrasonic frequency from 20 kHz to 20 + 40 kHz and then to 20 + 28 kHz, the sterilization rate first increases and then decreases. Simultaneously, a higher sterilization temperature leads to a higher sterilization rate. The surface slope of sterilization temperature is larger than that of ultrasonic frequency, indicating a more significant impact of sterilization temperature on sterilization rate. Figure 19 reveals that during the transformation of ultrasonic frequency from 20 kHz to 20 + 40 kHz and then to 20 + 28 kHz, the sterilization rate also follows an increasing and then decreasing pattern. Simultaneously, a longer processing time leads to a higher sterilization rate. The surface slope of processing time is larger than that of ultrasonic frequency, indicating a more significant impact of processing time on sterilization rate. Therefore, regarding the impact on sterilization rate, the order of significance is sterilization temperature > processing time > ultrasonic frequency.

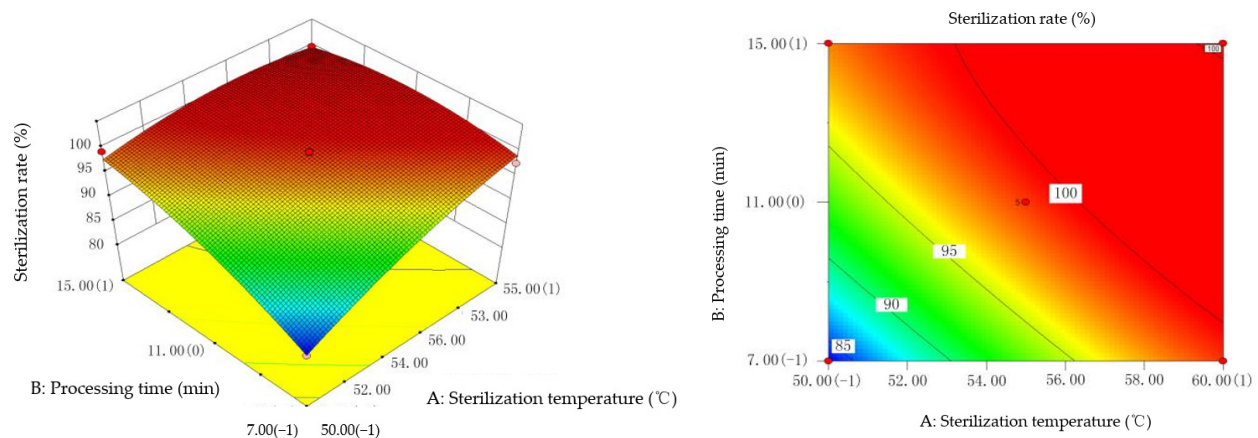


Figure 17. Response surface diagram of interaction between sterilization temperature and treatment time on sterilization rate.

Based on response surface experimental method, this section conducted experimental verification of the ultrasound–thermal synergistic sterilization control system designed in Section 2. Single-factor and response surface experiments were conducted on the commonly used synchronous synergistic sterilization function of the ultrasound–thermal synergistic sterilization control system, and the physicochemical properties of the sterilized liquid eggs were analyzed. Based on single-factor experiments, it was found that multi-frequency ultrasound improves sterilization efficiency by around 15% compared to traditional single-

source ultrasound. A three-factor, three-level experimental design was established, with the conclusion that the degree of influence is sterilization temperature > treatment time > ultrasonic frequency. Functional property tests on liquid eggs treated under different conditions revealed that the ultrasound–thermal synergistic sterilization control system has minimal impact on the foaming properties, viscosity, and other functional properties of liquid eggs.

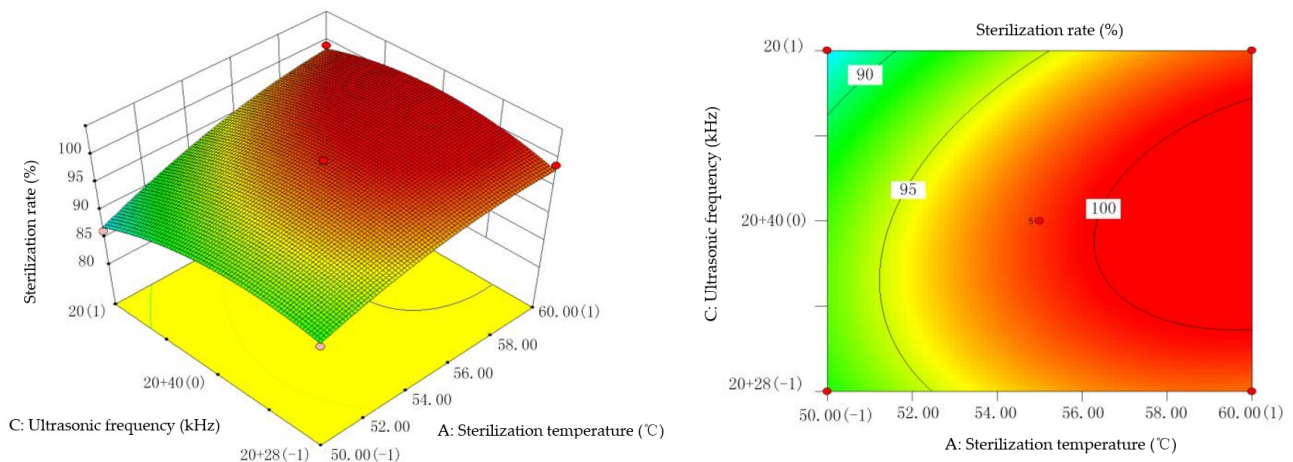


Figure 18. Response surface diagram of interaction between sterilization temperature and ultrasonic frequency on sterilization rate.

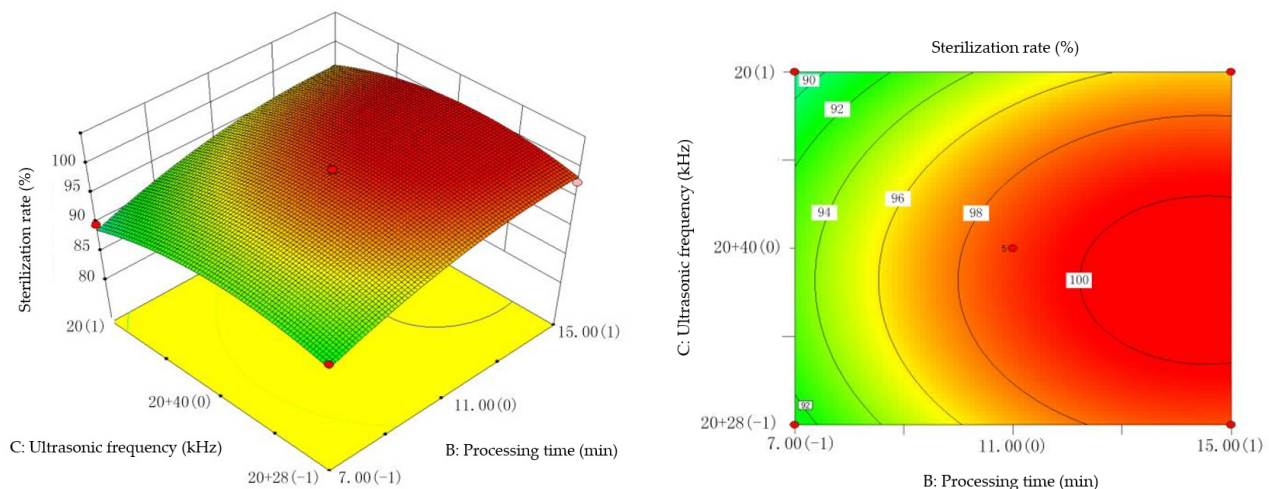


Figure 19. Response surface diagram of interaction between treatment time and ultrasonic frequency on bactericidal rate.

4. Sterilization Parameter Optimization

4.1. Principles of NSGA2 Genetic Algorithm

From the regression model and response surface analysis, it is evident that ultra sonic frequency, heat treatment temperature, and synergistic action time have different and mutually influential effects on sterilization rate and foaming properties. The method of parameter optimization using the Optimization function in Design-Expert 8.6 software during the response surface experiments resulted in significant deviations and insufficient accuracy. Therefore, this study employs the NSGA2 genetic algorithm for multi-objective parameter optimization to solve the regression model, which can make the parameter optimization results more accurate. In the NSGA2 algorithm, the sterilization rate and foaming properties of liquid eggs generated under all conditions of ultrasonic frequency, heat treatment temperature, and synergistic action time will undergo a unique non-dominated sorting.

Individuals with higher ranks (higher sterilization rate and foaming properties) will be inherited to obtain the optimal solution. Figure 20 represents the algorithm's computational flowchart.

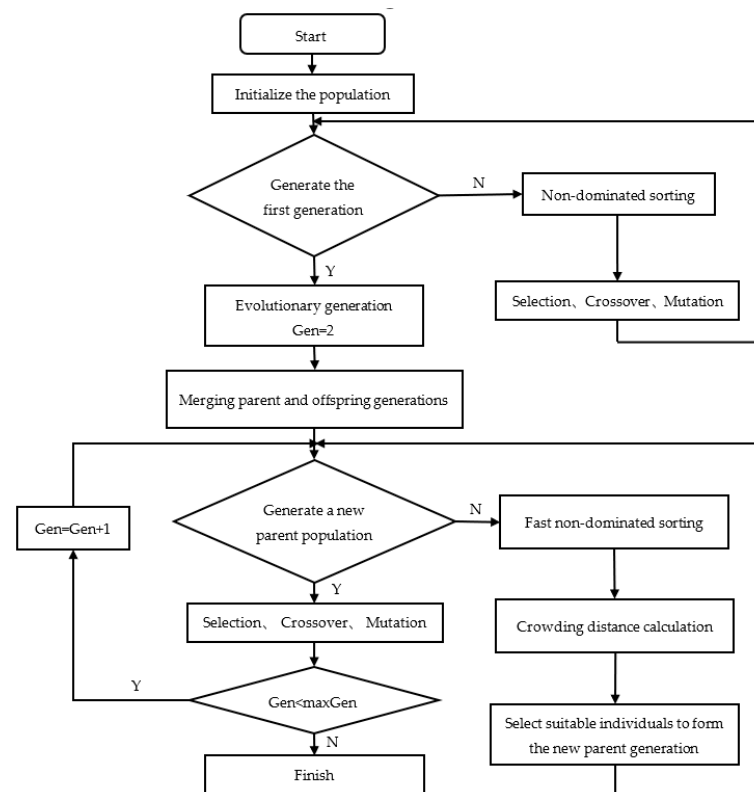


Figure 20. NSGA2 algorithm calculation flow chart.

4.2. Multi-Objective Modeling and Optimization Solution Based on Genetic Algorithm

In the process of ultrasonic–thermal synergistic sterilization, the sterilization parameters include sterilization temperature (A), processing time (B), and ultrasonic frequency (C). This paper selects several factors of ultrasonic–thermal synergistic sterilization as variables for the optimization model. The optimization variables can be represented as [A, B, C], and the objective function can be represented as [Y₁, Y₂]. Combining the prediction models for ultrasonic–thermal synergistic sterilization rate and foaming properties with the experimental parameters in Table 2, the mathematical model solved by the genetic algorithm in this study can be obtained as:

$$\begin{aligned}
 Y_1 &= -296.5721 + 10.6850A + 13.1559B - 15.1431C - 0.1694AB + 0.2535AC + 0.001875BC - 0.07219A^2 - 0.1325B^2 - 3.0448C^2 \\
 Y_2 &= -766.5755 + 29.0644A + 6.27175B - 0.0150C - 0.1375AB - 0.001875AC - 0.2673BC - 0.24478A^2 - 0.24478B^2 - 2.7990C^2 \\
 50 &\leq A \leq 60 \\
 7 &\leq B \leq 15 \\
 -1 &\leq C \leq 1
 \end{aligned} \tag{6}$$

Since A, B, and C take values within the process parameter range of ultrasonic–thermal synergistic sterilization, there are no nonlinear constraints. The optimization model only has inequality constraints. The multi-objective optimization function based on the genetic algorithm in MATLAB is used to solve the optimization model (NSGA2), with the optimization objectives being the maximum sterilization rate and foaming properties parameters. The crossover probability of NSGA2 genetic algorithm is set to 0.8, mutation probability to 0.05, and variable value step sizes for independent variables to 0.01, 0.01, and 1, respectively. The MATLAB program is used to obtain the Pareto optimal solution set. Through the algorithmic calculation analysis, combined with 50 sets of optimal solutions, the Pareto optimal solution set is obtained, as shown in Figure 21.

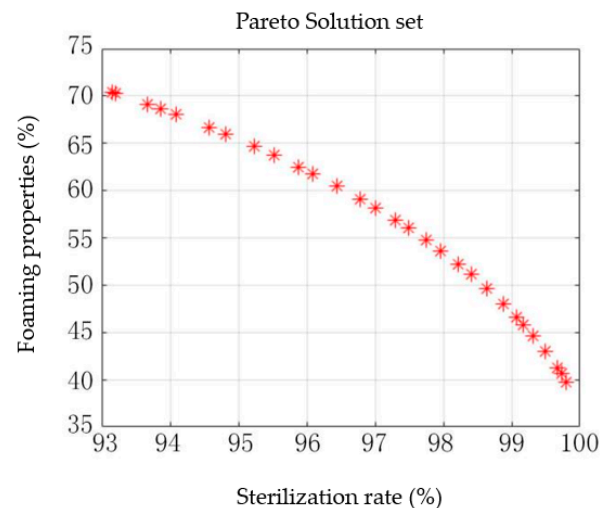


Figure 21. Pareto optimal solution set.

4.3. Parameter Optimization and Validation Experiments

MATLAB 2021 software is employed to run the NSGA2 algorithm for analysis and solution, with the optimal conditions being the maximum sterilization rate and foaming properties, under the premise of minimizing sterilization temperature and processing time to meet the requirements of sterilization rate and foaming properties. The calculated solution of the regression model yields the optimal parameters for ultrasonic–thermal synergistic liquid egg sterilization, as follows: sterilization temperature 55.11 °C, processing time 11.28 min, ultrasonic frequency 20 + 40 kHz. The predicted sterilization rate at these parameters is 99.87%, and the foaming properties are 45.80%.

To verify the reliability of the algorithm for parameter optimization, the sterilization temperature is adjusted to 55 °C, processing time to 11 min and 30 s, and ultrasonic frequency to 20 + 40 kHz, based on actual conditions. Three validation experiments are conducted under this process, with an average sterilization rate of 99.92% and foaming properties of 42.84%, which are within 5% difference from the theoretical values. This indicates that the equation fits well with the actual data, proving the effectiveness and feasibility of using genetic algorithms for parameter optimization. Additionally, measured physicochemical properties include foaming stability at 39.85%, viscosity at 37, and pH value at 7.15. Under the optimized sterilization conditions, microbial indicators comply with relevant national standards, and the impact on the functional properties of liquid eggs is minimal.

China requires pasteurization of liquid eggs at 64.5 °C for 3 min. The experimental parameters of the ultrasonic–thermal synergistic sterilization system obtained in this section, compared with the liquid eggs treated by the pasteurization method [25], show that the functional properties of liquid eggs treated by the ultrasonic–thermal synergistic sterilization method are superior to pasteurization, while ensuring sterilization effectiveness. The comparison chart of the impact on the functional properties of liquid eggs between the ultrasonic–thermal synergistic sterilization control system and commercially available pasteurized liquid eggs is shown in Figure 22, with specific data presented in Table 7. From Figure 22a, it can be seen that the processing temperature of traditional pasteurization for liquid eggs is higher than untreated and ultrasonic–thermal synergistic sterilization control system processing temperatures. Figure 22b shows that pasteurization has a negative effect on the foaming properties of liquid eggs, while ultrasonic–thermal synergistic sterilization treatment can increase the foaming properties, leading to better results when making products such as bread and egg tarts using liquid eggs. Figure 22c shows that the viscosity of liquid eggs significantly increases after treatment, and the viscosity after ultrasonic–thermal synergistic sterilization treatment is lower than after pasteurization. Analysis indicates that compared to pasteurization, the ultrasonic–thermal synergistic sterilization treatment

results in larger gelatinous gaps, a more dispersed structure, and changes in the gel-like network and denaturation of proteins, altering the viscoelastic properties of proteins [26]. The viscosity of liquid eggs caused by pasteurization is high, and the process is prone to condensation and blockage in the sterilization equipment pipeline, significantly reducing the foaming properties. In summary, compared to pasteurization technology, the application of ultrasonic–thermal synergistic sterilization technology for liquid egg sterilization has significant advantages. It can be seen that using response surface analysis and NSGA2 genetic algorithm can achieve optimization of liquid egg sterilization parameters under multiple objectives, ensuring food safety while minimizing the impact of sterilization on the functional properties of liquid eggs. This results in liquid egg sterilization process parameters with practical application value.

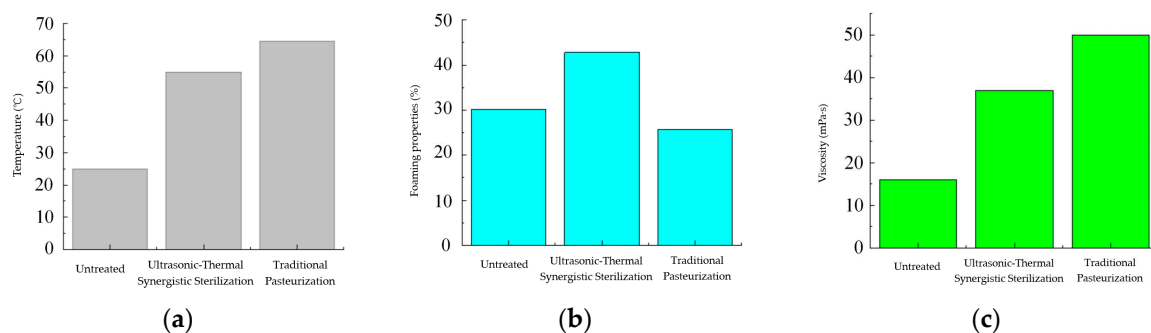


Figure 22. Comparison between ultrasonic–thermal synergistic sterilization control system and pasteurization: (a) comparison chart of processing temperature; (b) comparison chart of foaming properties; (c) comparison chart of viscosity properties.

Table 7. Experimental comparison results.

	Test Value				Model Predicted Values	Model Error
	Test Group 1	Test Group 2	Test Group 3	Test Mean		
Lethal logarithm	3.15	3.30	3.05	3.17	-	-
Sterilization rate/%	99.93	99.95	99.91	99.93	99.92	0.16
Foaming properties/%	43.88	43.25	41.38	42.84	45.80	3.01

In this section, the implementation process of the proposed multi-objective optimization algorithm was initially introduced. The NSGA2 algorithm was utilized to solve the regression equations obtained from the response surface experiments, calculating the Pareto optimal solution set and filtering the solutions. It was found that, at a sterilization temperature of 55 °C, treatment time of 11 min and 30 s, and ultrasonic frequency of 20 + 40 kHz, the foaming properties of liquid eggs could be maximized while ensuring the required sterilization rate. A comparison revealed that the functional properties of liquid eggs processed with the ultrasound–thermal synergistic sterilization control system were superior to those of traditionally pasteurized eggs. The final experimental comparison results indicated an average sterilization rate of 99.93%, an average decimal reduction of 3.17 log values, and a foaming property of 42.84%. The optimal conditions for ultrasonic–thermal synergistic sterilization were determined through experimental results, with a sterilization temperature of 55 °C, a processing time of 11 min and 30 s, and the application of ultrasound at a frequency of 20 + 40 kHz. Under these conditions, the average total sterilization rate reached 99.93%, meeting the requirements of the National Standard for Food Safety “Eggs and Egg Products” GB 21710-2016 [27], which specifies that the microbial count in sterilized liquid eggs should be less than 100. The calculated sterilization rate required to meet this standard is 99.15%, and the obtained sterilization rate surpasses this threshold, thus satisfying the national standard.

5. Conclusions

This paper innovatively proposes a multi-frequency ultrasound–thermal synergistic sterilization method. Through COMSOL multi-physics simulation and sterilization experiments, the effects of multi-frequency combinations and thermal treatment parameters on sterilization performance and functional properties are explored. The optimal process parameters are determined as follows: ultrasound frequencies of 20 kHz + 40 kHz, treatment time of 11 min and 30 s, and heating temperature of 55 °C. In actual ultrasound–thermal synergistic sterilization experiments, a sterilization rate of 99.32% and a foaming property of 42.79% are achieved. The results indicate that, while ensuring sterilization performance, the method avoids significant damage to the functional properties of liquid eggs caused by the intense heat treatment of traditional pasteurization processes. The authors also hope that the ultrasonic sterilization system studied in this paper can be further technologically optimized and widely applied in other industries in the future. The conclusions are as follows:

(1) Utilizing COMSOL multi-physics field simulation, the acoustic pressure propagation attenuation of a single-frequency ultrasonic transducer in liquid egg medium was simulated. The results indicate that the horizontally covered distance of the 20 kHz ultrasonic transducer is approximately 200 mm, and the vertically propagated distance is about 100 mm. Based on this, a multi-frequency ultrasonic transducer arrangement scheme was designed, and a simulation of the multi-frequency ultrasonic field distribution was conducted. The results show that the designed multi-frequency sterilization scheme is reasonable. The simulated average sound pressure of ultrasonic waves ranges from 2.2 to 3.8 Pa. Through sound intensity detection experiments, it is verified that the required sound intensity effect for liquid egg sterilization can be achieved. A design for an ultrasonic–thermal synergistic liquid egg sterilization scheme and apparatus was formulated, and a control system based on the Modbus communication protocol was established. Through testing, the effectiveness of the control, data acquisition, and processing functions of the ultrasonic–thermal synergistic sterilization device were validated.

(2) Sterilization rate and foaming properties were used as evaluation indicators. Single-factor screening was conducted for ultrasonic frequency, action time, and heating temperature. Furthermore, a three-factor, three-level response surface experiment was carried out for ultrasonic frequency, action time, and heating temperature. Based on the variance analysis of the regression model, it was found that ultrasonic frequency has a significant correlation with the sterilization rate. The results indicate a strong correlation between ultrasonic frequency, action time, heating temperature, and sterilization performance. Multi-frequency ultrasound improves sterilization efficiency by approximately 15% compared to traditional single-source ultrasonic sterilization. The order of impact on sterilization rate was found to be: sterilization temperature > action time > ultrasonic frequency, demonstrating that the ultrasonic–thermal synergistic sterilization system has good sterilization effects on liquid eggs.

(3) Sterilization rate and foaming properties were optimized objectives using a genetic algorithm for multi-objective optimization. Combined with the response surface regression model, the following optimal process parameters were obtained: ultrasonic frequency of 20 kHz + 40 kHz, action time of 11 min and 30 s, and heating temperature of 55 °C. The predicted sterilization rate was 99.92%, and the foaming property was 45.80%. In actual ultrasonic–mild thermal synergistic sterilization experiments, the sterilization rate was 99.93%, there was an average decimal reduction of 3.17 log values, and the foaming property was 42.84%. Compared with national standards and traditional pasteurization, the ultrasonic–thermal synergistic sterilization control system ensures the quality of liquid eggs based on indicators such as foaming properties and viscosity while meeting national sterilization requirements.

The sterilization method and experiments proposed in this paper have not been applied to actual liquid egg production lines, and further work will involve the optimization and integrated application of ultrasonic–thermal synergistic sterilization equipment. In

the future, this system could be promoted for use in fields such as food safety, maritime or medical industries for microbial contamination control, and drinking water sterilization.

Author Contributions: All authors contributed substantially to this work. W.F., conceptualization, supervision, validation, writing-review, and editing; H.C., methodology and writing-original draft preparation; H.L., formal analysis; L.M., software and data curation; Q.W., investigation. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key Research and Development Program of China (Program No. 2022YFD1300705) and the Fundamental Research Funds for the Central Universities (Program No. 2662023GXPY005).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The datasets generated for this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Wang, J.; Xu, L.; Lv, Y.; Su, Y.; Gu, L.; Chang, C.; Zhang, M.; Yang, Y.; Li, J. To improve the gel properties of liquid whole egg by short-term lactic acid bacteria fermentation. *Innov. Food Sci. Emerg. Technol.* **2022**, *75*, 102873. [\[CrossRef\]](#)
2. Huang, L.; Wang, T.; Han, Z.; Meng, Y.; Lu, X. Effect of egg yolk freezing on properties of mayonnaise. *Food Hydrocoll.* **2016**, *56*, 311–317. [\[CrossRef\]](#)
3. Jin, H.; Pan, J.; Zeng, Q.; Li, Z.; Jin, Y.; Sheng, L. Competitive adsorption of binary negatively charged proteins in egg white during foam evolution: From bulk solution to air-water interface. *Food Hydrocoll.* **2023**, *140*, 108680. [\[CrossRef\]](#)
4. Puertas, G.; Vázquez, M. Liquid whole egg fractionation: Effect of centrifugation on physicochemical attributes of quality. *J. Food Process. Preserv.* **2021**, *45*, e15334. [\[CrossRef\]](#)
5. Kim, Y.J.; Moon, H.J.; Lee, S.K.; Song, B.R.; Lim, J.S.; Heo, E.J.; Park, H.J.; Wee, S.H.; Moon, J.S. Development and validation of predictive model for Salmonella growth in unpasteurized liquid eggs. *Korean J. Food Sci. Anim. Resour.* **2018**, *38*, 442. [\[PubMed\]](#)
6. Jin, H.; Sun, Y.; Pan, J.; Fang, Y.; Jin, Y.; Sheng, L. Adsorption kinetics of ovalbumin and lysozyme at the air-water interface and foam properties at neutral pH. *Food Hydrocoll.* **2022**, *124*, 107352. [\[CrossRef\]](#)
7. Foegeding, P.M.; Stanley, N.W. Growth and inactivation of microorganisms isolated from ultrapasteurized egg. *J. Food Sci.* **1987**, *52*, 1219–1227. [\[CrossRef\]](#)
8. Marco-Molés, R.; Rojas-Graü, M.A.; Hernando, I.; Pérez-Munuera, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Physical and structural changes in liquid whole egg treated with high-intensity pulsed electric fields. *J. Food Sci.* **2011**, *76*, C257–C264. [\[CrossRef\]](#)
9. Ahmed, T.A.; Younes, M.; Wu, L.; Hincke, M.T. A survey of recent patents in engineering technology for the screening, separation and processing of eggshell. *Front. Bioeng. Biotechnol.* **2021**, *9*, 677559. [\[CrossRef\]](#)
10. Long, H.; Nie, L.; Xiang, X.; Li, H.; Zhang, X.; Fu, X.; Ren, H.; Liu, W.; Wang, Q.; Wu, Q. Effects of Ultrahigh Temperature Pasteurization on the Liquid Components and Functional Properties of Stored Liquid Whole Eggs. *BioMed Res. Int.* **2020**, *2020*, 1–10.
11. Geveke, D.J.; Bigley AB, W.; Brunkhorst, C.D. Pasteurization of shell eggs using radio frequency heating. *J. Food Eng.* **2017**, *193*, 53–57. [\[CrossRef\]](#)
12. Yan, W.; Xu, B.; Jia, F.; Dai, R.; Li, X. The effect of high-pressure carbon dioxide on the skeletal muscle myoglobin. *Food Bioprocess Technol.* **2016**, *9*, 1716–1723. [\[CrossRef\]](#)
13. Hermawan, N.; Evrendilek, G.A.; Dantzer, W.; Zhang, Q.; Richter, E. Pulsed electric field treatment of liquid whole egg inoculated with Salmonella enteritidis. *J. Food Saf.* **2004**, *24*, 71–85. [\[CrossRef\]](#)
14. Yuk, H.G.; Geveke, D.J.; Zhang, H.Q. Efficacy of supercritical carbon dioxide for nonthermal inactivation of Escherichia coli K12 in apple cider. *Int. J. Food Microbiol.* **2010**, *138*, 91–99. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Paniwnyk, L. Applications of ultrasound in processing of liquid foods: A review. *Ultrason. Sonochem.* **2017**, *38*, 794–806. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Cao, X.; Zhang, M.; Mujumdar, A.S.; Zhong, Q.; Wang, Z. Effects of ultrasonic pretreatments on quality, energy consumption and sterilization of barley grass in freeze drying. *Ultrason. Sonochem.* **2018**, *40*, 333–340. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Dai, J.; Bai, M.; Li, C.; Cui, H.; Lin, L. Advances in the mechanism of different antibacterial strategies based on ultrasound technique for controlling bacterial contamination in food industry. *Trends Food Sci. Technol.* **2020**, *105*, 211–222. [\[CrossRef\]](#)
18. Mantas, P.; Pagán, R.; Raso, J. Predicting lethal effect of ultrasonic waves under pressure treatments on Listeria monocytogenes ATCC 15313 by power measurements. *J. Food Sci.* **2000**, *65*, 663–667. [\[CrossRef\]](#)
19. Huang, E.; Mittal, G.S.; Griffiths, M.W. Inactivation of Salmonella enteritidis in liquid whole egg using combination treatments of pulsed electric field, high pressure and ultrasound. *Biosyst. Eng.* **2006**, *94*, 403–413. [\[CrossRef\]](#)
20. Li, J.; Ding, T.; Liao, X.; Chen, S.; Ye, X.; Liu, D. Synergetic effects of ultrasound and slightly acidic electrolyzed water against Staphylococcus aureus evaluated by flow cytometry and electron microscopy. *Ultrason. Sonochem.* **2017**, *38*, 711–719. [\[CrossRef\]](#)

21. Lee, Y.; Kim, H.; Baek, M. Research on ultrasonic sterilization effect of treatment equipments. *J. Korean Soc. Manuf. Technol. Eng.* **2013**, *22*, 818–823.
22. Li, M.; Zhou, C.; Wang, B.; Zeng, S.; Mu, R.; Li, G.; Li, B.; Lv, W. Research progress and application of ultrasonic-and microwave-assisted food processing technology. *Compr. Rev. Food Sci. Food Saf.* **2023**, *22*, 3707–3731. [[CrossRef](#)] [[PubMed](#)]
23. Patrignani, F.; Vannini, L.; Kamdem, S.L.S.; Hernando, I.; Marco-Molés, R.; Guerzoni, M.E.; Lanciotti, R. High pressure homogenization vs heat treatment: Safety and functional properties of liquid whole egg. *Food Microbiol.* **2013**, *36*, 63–69. [[CrossRef](#)] [[PubMed](#)]
24. Rita de Cássia, S.S.; Coimbra, J.S.; Rojas, E.E.G.; Minim, L.A.; Oliveira, F.C.; Minim, V.P. Effect of pH and salt concentration on the solubility and density of egg yolk and plasma egg yolk. *LWT Food Sci. Technol.* **2007**, *40*, 1253–1258.
25. Ho, H.Y.; Ciou, J.Y.; Qiu, Y.T.; Hsieh, S.L.; Shih, M.K.; Chen, M.H.; Tu, C.W.; Hsieh, C.W.; Hou, C.Y. Improvement of foaming characteristics and stability of sterilized liquid egg with egg white hydrolysate (EWH). *Foods* **2021**, *10*, 1326. [[CrossRef](#)]
26. Balekelayi, N.; Woldehellasse, H.; Tesfamariam, S. Comparison of the performance of a surrogate based Gaussian process, NSGA2 and PSO multi-objective optimization of the operation and fuzzy structural reliability of water distribution system: Case study for the City of Asmara, Eritrea. *Water Resour. Manag.* **2022**, *36*, 6169–6185. [[CrossRef](#)]
27. GB 27170-2016; National Food Safety Standard-Hygienic Specifications for Egg and Egg Products Production. National Health and Family Planning Commission of the People's Republic of China, China Food and Drug Administration, 2016. Available online: <https://faolex.fao.org/docs/pdf/chn215941.pdf> (accessed on 19 March 2024).

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.