Weibull Kinetic Modeling and Nutritional Effects of High-hydrostatic-pressure Sterilization of Soft-packing Boiled Bamboo Shoots

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Abstract Application of high-hydrostatic-pressure (HHP) sterilization technology to boiled bamboo shoots was preliminarily discussed. An improved Weibull model that considered pressure and time as independent variables simultaneously for simulating the sterilization result was obtained by fitting the microbial lethal curves with high precision ($R^2 > 0.98$). HHP sterilization parameters could be calculated using this model with a certain disinfection rate. For a sterilization rate of 99.5%, treatment with a pressure of 400 MPa for 6 min or 500 MPa for 3 min at room temperature could exterminate all pathogenic microorganisms in boiled bamboo shoots, and control the total number of colonies at a low level below 100 CFU/g. Comparisons of the effects of HHP, high-pressure steam and microwave on the softness and contents of soluble protein and vitamin C of samples were also made. The results showed that the extent of damage of the products' original quality caused by HHP sterilization was less than that caused by the thermal and microwave treatment processes with equivalent sterilization percentages.

Keywords: HHP, sterilization, boiled bamboo shoots, Weibull model, nutrition

Introduction

Bamboo shoots are China's characteristic agricultural product, and they have been well known as a "Healthy Food" because of their delicious taste and rich nutrient profile consisting of soluble protein and various vitamins. Furthermore, bamboo shoots can warm the stomach, invigorate the spleen and help people to remain slim (1). Recently, with the acknowledgment of the medicinal and edible value of bamboo shoots, global consumption of this product is growing 15% every year (2). Bacteria in bamboo shoots are still the main cause of safety and quality problems, especially contamination during the packaging process. Because of the low level of automation and food safety awareness, many products are produced manually and lack sterilization after packaging. This is a critical issue and it leads to diseases and damage (3). Many sterilizing methods have been studied and applied to eliminate bacteria after the packaging process.

However, the traditional thermal sterilization process can easily cause undesirable nutrition loss and damage the sensory quality of this product. Only 55% of the vitamin C in pineapple juice remained after treatment at 73°C for 15 min (4). The soluble protein content

and flavor also decreased after thermal sterilization (5). High-pressure steam (high-temperature steam) and microwave processing caused less damage to the nutrition than other thermal treatments due to their short processing time, however, there was still some reduction of the soluble protein and vitamin C contents (6,7). Furthermore, water loss and overheating still exist in all of these processes (8). High-hydrostatic-pressure (HHP) processing is a non-thermal method that can effectively sterilize and maintain the original nutrients and flavor of food. This technique has already become a most major topic of discussion in the world (9,10).

Currently, studies of the HHP technique mainly focus on beverages and jam products, while there has been much less research on typical solid foods such as bamboo shoots. Modified atmosphere packaging (MPA) and the fermentation process of bamboo shoots have been discussed. The quality and production process of soft packaged boiled bamboo shoots has concerned Chinese scholars since the 1990s. Almost all of the flavor and nutrient substances are better retained with HHP than with thermal sterilization. HHP could exterminate microorganisms in cane shoots at room temperature without causing any nutrition loss (11). The flavor of asparagus and the amino acid content of fresh bamboo shoots had little to no

change after HHP in similar studies (12). HHP sterilization can effectively maintain the color, flavor and nutrients of food, but the high temperature and high pressure can promote the oxidation of vitamin C, causing loss of the vitamin. Research showed that 85% of the vitamin C content of kiwi juice remained after HHP processing at normal temperatures (13). Although there have been many studies of HHP sterilization technology, there has not been enough application of this technique to bamboo shoots.

Experiments on the sterilization effect of one product are not enough for commercial production. Kinetic models can conveniently predict the sterilization results and can provide theoretical direction for industrial applications. Kinetic models can be obtained by plotting the logarithm of the number of survivors against exposure time. First-order kinetics (14), log-logistic equations (15), the Weibull model (16) and a response surface model (17) have been examined and used to predict the sterilization effect on many types of bacteria and liquid foods (18). The Weibull model is widely used due to its simple and flexible characteristics. It has been successfully applied to model the thermal inactivation of *Salmonella enterica serovar typhimurium* (19) and HHP sterilization of *Listeria monocytogenes* (20), *Bacillus cereus* spores in milk (21) and natural microbiota in pineapple puree (22). These models are often obtained as a group of equations and mostly cannot express the continuous change in pressure. Furthermore, relevant model applications for solid foods such as bamboo shoots are still limited. Log-logistic and response surface models have more parameters than others and are intrinsically complex, but the first-order kinetics are less accurate. This study aims to establish a single model that can describe continuous pressure changes, so a Weibull model is used, although other models may also yield accurate results (15–18).

This paper focused on evaluating the sterilization effects of the HHP process on soft-packed boiled bamboo shoots and establishing and improving a microbial lethal prediction model based on the Weibull model. The softness of boiled bamboo shoots and the contents of soluble protein and vitamin C after HHP, high-pressure steam and microwave sterilization were compared in order to provide theoretical support for this process.

Materials and Methods

Unsterilized boiled bamboo shoots and PA/PE bags (10×10 cm, 85 µm) were provided by Fuyang Bamboo Technology Co., Ltd. (Zhejiang, China); Plate Count Agar (BR) and NaCl (AR) were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Bamboo shoots were precooked at 100°C for 1 h and cut into similar pieces weighing 10±0.5 g each and then vacuum-sealed in PA/PE bags without sterilization. Bamboo shoots were contaminated by bacteria during the cutting and packaging process, and their concentration was approximately 2.2×10⁴ CFU/g. Samples were stored at 4°C for further use.

Table 1. Experimental plan of HHP treatment

Pressure (MPa)		Pressure holding time (min)					
200	1	2	3	6	9	14	20
300	1	2	3	6	9	14	20
400	1	2	3	6	9	14	20
500	1	2	3	6	9	14	20

Treatment of sterilization HHP treatments (model 600 MPa/5 L) were performed with a high hydrostatic pressure device (using water as the pressure transmitting medium) provided by Baotou Kefa High Technology Co., Ltd. (Neimengu, China). Several pressure levels (200, 300, 400 and 500 MPa) with different pressure holding times (1, 2, 3, 6, 9, 14 and 20 min) were applied to unsterilized boiled bamboo shoots at room temperature, as shown in Table 1. Microbiological analysis and model establishment were based on this step.

Microwave and high-pressure (temperature) steam treatments were applied to unsterilized samples to compare the nutrition loss from these treatments to that of HHP-treated samples with an equivalent sterilization percentage (99.5%).

Microwave sterilization was carried out using a microwave oven (model NJL07-3) provided by Nanjing Jiequan Microwave Equipment Co., Ltd. (Jiangsu, China). Microwave power, time and the sample's temperature can be well monitored with this device. According to Pang Mengyao's research, samples were sterilized under 600 W microwave power for 103 s (23).

High-pressure (temperature) steam sterilization was performed with an autoclave provided by Shanghai Shenan Medical Instrument Factory (Shanghai, China). Unlike HHP treatment (more than 100 MPa, room temperature), the autoclave provided a relatively higher pressure (0.1 MPa) than a standard atmosphere at 125°C. On the basis of Xu Jixiang's study, the samples were treated at 115°C for 15 min (7).

Microbiological analysis Each bag of sample (10 g) was mixed with 90 mL of sterilized physiological saline and homogenized into a liquid state aseptically after sterilization. A series of dilutions was performed in strict accordance with the national standard (24). The total bacterial count was enumerated (CFU/g) by plating 1 mL of the liquid sample on PCA medium incubated for 48 h at 37°C.

Model fitting and improvement The Weibull distribution model has been used to fit microorganism lethal process data in the literature. It was shown that the Weibull model had greater accuracy than the first-order kinetics equation (25,26). The pressure holding time for a specific microorganism lethal rate can be calculated from this model:

$$\log \frac{N}{N_0} = -bt^n \quad (1)$$

where N is microbial residues in food after a period of sterilization time (CFU/g), N_0 is the original bacterial content in food (CFU/g), b is

a size parameter, n is a shape parameter, and t is the pressure holding time (min).

Recent models cannot show the effect of continuous pressure changes on the HHP process; the Weibull model can demonstrate this effect. A group of Weibull models can be fitted and established under different pressure levels. This paper will attempt to combine these models into one. The b and n values have a significant relationship with the pressure, and an improved model can be obtained by using a pressure value to express the two parameters. The new model will more conveniently predict the sterilization effect of HHP on boiled bamboo shoots and similar products.

Quality analysis Softness, soluble protein and vitamin C content are the main quality factors of bamboo shoots. Softness (kg/cm^2) measurement was carried out with a fruit hardness tester provided by Hangzhou Tuopu Instrument Co. Ltd. (Hangzhou, China).

Soluble protein (mg/g) analysis was performed according to the method described by Bradford (27).

Vitamin C ($\text{mg}/100 \text{ g}$) analysis was performed according to the national standard (28). All of the samples were chosen from the same part of different bamboo shoots and analyzed immediately after the sterilization process.

Statistical analysis All results were reported as the means of three independent bags of boiled bamboo shoots, and each sample was analyzed in triplicate. Data analysis was performed using Matlab software.

Results and Discussion

Microbiological analysis High pressure can damage the cell wall and membrane, affect the balance of the internal biochemical reaction, and result in the death of microorganisms. Due to the existence of the tailing phenomenon and pressure-resistant bacteria (gram-positive bacterium), a small amount of residual bacteria remained (29). Figure 1 shows the relationship between the bacterial survival rate and pressure holding time at different pressures.

As shown in Fig. 1, the total number of colonies decreased significantly after HHP treatment. As expected, pressure had great lethality and reduced the microbial load. The survival count for each pressure condition of microbes decreased over time. At the same pressure level, extension of the pressure holding time increased the sterilization effect gradually, and the total number of colonies in the samples decreased; a more than 2 log reduction could be detected as time increased under the 300, 400, 500 MPa conditions, but the 200 MPa condition had little lethality (less than 1 log) to microbes due to its low intensity. Beyond a certain range, increasing the holding time had no significant enhancement of the sterilization effect; microbial residues in the 400 MPa condition decreased only 0.1 log when the time was changed from 14 to 20 min, and a similar phenomenon was

Fig. 1. Microorganism lethal curves of soft-packed boiled bamboo shoots during HHP sterilization with different pressures

seen at other pressure levels. The sterilization effect could be promoted by increasing the pressure. The bacterial survival rate could be reduced dramatically by raising the pressure and lengthening the holding period, to a point. The total colony number declined sharply by two orders of magnitude. The inactivation rates at 400 and 500 MPa were similar. In this case, an increase in pressure could not exterminate residual bacteria completely. HHP sterilization still met the requirement of the national standards for the commercial sterilization level of food (30). After the HHP treatment of 400 MPa for 6 min, the total colony number was reduced to below 100 CFU/g. The sterilization rate was 99.6%; *Escherichia coli* and other pathogens were not detected.

Model fitting and improvement The Weibull model is widely used to simulate the sterilization of HHP because of its simple and flexible characteristics (20-23) and can accurately describe non-linear lethal curves. Based on the experimental data, model fitting was carried out using Matlab software. The fitting results are shown in Fig. 2.

As shown in Fig. 2, the Weibull model was in good agreement with experimental data. The sterilization rates of different types of microorganisms under HHP treatment in the samples were not the same because of the different resistance and sensitivity of microorganisms to high pressure. The R^2 values of the four pressure conditions were 0.9843, 0.9981, 0.9946, and 0.9967, respectively; the RMSE values were small. The results indicated that using the Weibull model to describe the death of microorganisms in soft-packed boiled bamboo shoots is extremely accurate. Therefore, the Weibull model can simulate the microbial inactivation curve and predict the sterilization rate of this product. The Weibull equations of different pressures are shown in Table 2.

The b parameter is a scale factor related to the microbial inactivation rate. As seen in Table 2 and Fig. 2, the b value of the model increases gradually with an increase in pressure; it increased from 0.2267 to 2.039 as the pressure increased from 200 to 500 MPa. Similar results

Fig. 2. Fitting curves using Weibull model for HHP sterilization with different pressure

Table 2. Weibull kinetic equations for HHP sterilization with different pressure

pressure/MPa	Weibull kinetic equations	R^2	RMSE
200	$\log \frac{N}{N_0} = -0.2267t^{0.5284}$	0.9843	0.051
300	$\log \frac{N}{N_0} = -1.383t^{0.1776}$	0.9981	0.035
400	$\log \frac{N}{N_0} = -1.866t^{0.1297}$	0.9946	0.069
500	$\log \frac{N}{N_0} = -2.039t^{0.1167}$	0.9967	0.058

were found in Daryaei's research (31), where the b value increased from 0.81 to 1.66 as the pressure increased during the inactivation of *Bacillus cereus* spores in cooked rice. Different types of bacteria might lead to different b values (32). A greater sterilization effect yielded a higher b value, proving that the size parameter b has a close relationship with sterilization strength. However, the b values here were non-linear and were different from Evelyn's results, which showed linear b values in the HHP treatment of psychotropic *Bacillus cereus* spores in milk (21). These differences are mainly due to the variety of food and bacteria; a model for a certain product might not be suitable for all products. Despite this caveat, this research method still has great significance and warrants further study (33,34).

The shape parameter n represents the deviation of the survival curves from linearity. As shown in Table 2 and Fig. 2, the n values of the equations were all less than 1 and revealed a concave upward curve of lethality. This is because of the complex resistance of the microbial population to lethal treatment (17,35). The sensitive microbial population was eliminated quickly at first, and the more resistant population showed a slower and steady decline (36). With the extension of the pressure holding time, the lethal curve showed

Table 3. Relationship between pressure and the model's parameters

Model's parameters	Pressure (MPa)			
	200	300	400	500
b	0.2267	1.383	1.866	2.039
n	0.5284	0.1776	0.1297	0.1167

a tailing phenomenon. The shape parameter n reflected the tailing extent (37); a smaller n value would result in a more concave curve. Under 200 MPa of pressure, the n value was the largest because the death rate of bacteria was slow. When the pressure was gradually increased to 500 MPa, the death rate increased and the n value decreased, causing a quick appearance of tailing with the extension of the pressure holding time (33). The n values here ranged from 0.1167 to 0.5284 and were also non-linear like the b values. These two parameters were related to the pressure, and similar conclusions were also reported by Juliano (38) and Buzrul (32).

As shown in Table 3, the b and n values were closely related to the pressure, and once the functional relationship was found, all of the equations of different pressures could be integrated into one. This new model could reveal the effect of the pressure holding time as well as the pressure level and could be used for more convenient predictions. The relationship between pressure and the two parameters was as follows:

The relationship could be fitted, referring to Chen and Hoover's study (18); in his research, if the n value could be approximately constant during the pressure inactivation of *Yersinia enterocolitica* in milk (400-500 MPa) and buffer (300-400 MPa), then the scale factor b could be fitted to a linear equation. Similar linearity was also reported by Evelyn (21), as mentioned above. However, there were obviously some differences. The n value could not be seen as a constant, and the b value and pressure were nonlinearly related ($R^2 < 0.88$). In a certain short range of pressure or temperature, the b and n parameters might be linear, but not over a wide range. This is because food sterilization is a complex process. Different food characters (solid or liquid, acid or neutral) and ingredients (protein or sugar) all have significant effects on sterilization, so a model for one type of food might not suit another. These models still have reference significance for other foods, especially this method, which could also be applied to other studies.

To improve the Weibull model to reveal the pressure level more clearly, the b and n values could be fitted to the following equation:

$$y = -a \exp(-cP) + d \quad (2)$$

where P is the pressure in the sterilization process and a , c , and d are constants.

Curve fitting could be performed with Matlab software; the equations are listed in Table 3. The results showed that b and n could be well fitted to equation (2) ($R^2 > 0.99$). Pressures below 200 MPa and above 500 MPa were not discussed here because this paper is focused on the HHP sterilization of boiled bamboo shoots at room

temperature. Low pressure had no effect on microorganisms, and a much higher pressure did not enhance the sterilization due to the tailing effect. The holding time was limited below 20 min for the same reason. The fitting equations were as follows:

$$b = -12.01\exp(-0.0019P) + 2.171 \quad (3)$$

$$n = 18.84\exp(-0.01913P) + 0.1177 \quad (4)$$

By substituting the fitting equations of b and n into Eq. (1), we can obtain the improved Weibull model. This new model could predict the survival of microorganisms at pressure levels between 200 and 500 MPa. The whole model is as follows:

$$\log \frac{N}{N_0} = [12.01\exp(-0.0091P) - 2.171]t^{18.84\exp(-0.01913P) + 0.1177} \quad (5)$$

Model verification The model was verified by a group of tests, with pressures between 200 and 500 MPa and holding times between 1 and 20 min. The measured values and the predicted values were fitted using $y=ax$; $\log$ values of residual microorganisms after HHP treatment in the boiled bamboo shoots were used as the ordinate values, and the predicted values of the improved Weibull model were used as the abscissa. As shown in Fig. 3, the measured values and the predicted values were well fitted ($y=1.006x$, $R^2=0.9869$). Thus, the improved Weibull model of HHP sterilization for soft-packed boiled bamboo shoots had similar accuracy to previous studies (32-40) and could be used to predict the sterilization rate of these products.

According to the improved Weibull model and the commercial sterilization level, HHP process parameters could be chosen and calculated easily. When setting the sterilization rate to 99.5%, the $\log(N/N_0)$ should be -2.301 ; then, the HHP treatments chosen to follow the research would be 400 MPa for 6 min and 500 MPa for 3 min. The results at 200 and 300 MPa did not match the standard in a short time.

Quality analysis The softness of bamboo shoots directly reflected the sterilization damage to the structure. Bamboo shoots had a softness of 7.2 kg/cm^2 before sterilization. After sterilization, the samples lost water and had decreased softness, as shown in Fig. 4A. One reason for this finding is that high temperature or high pressure broke the cells of the bamboo shoots, causing the loss of cytosol, and softened the shoot structure (39). Another reason is that the bamboo shoot proteins denatured and formed a gel after dehydration (40), decreasing the softness of the bamboo shoots. Samples treated with high-pressure steam had the lowest softness (6.3 kg/cm^2) because of the long treatment time (15 min). HHP seemed to be the best way to maintain the softness of bamboo shoots, although it did cause a slight decrease (0.2 for 400 MPa, 0.4 for 500 MPa) in softness compared to the microwave (0.6) and high-pressure steam processing (almost 1) methods.

Soluble protein is a sensitive component in food. Food with a high

Fig. 3. Correlation between the measured values and the predicted values

protein content could easily suffer a decay in quality after high-temperature sterilization (41). At an ultra-high level ($>700 \text{ MPa}$), pressure can damage the secondary structure of protein and cause irreversible denaturation (42). It is necessary to discuss the effect of thermal and HHP sterilization on the original protein content of boiled bamboo shoots. As shown in Fig. 4B, the soluble protein of all of the samples decreased. Thermal sterilization damaged the primary structure of the protein, causing irreversible crosslinking and dissociation and reducing the solubility, which decreased the soluble protein content. The decrease in the soluble protein content after high-pressure steam sterilization was 12.20%. Microwave sterilization had a smaller influence on soluble protein because of the short processing time. HHP sterilization ($<500 \text{ MPa}$) has a small effect on the soluble protein; the decrease was probably due to the loss of soluble protein in the water extruded by the pressure.

Vitamin C was undoubtedly the most sensitive ingredient in boiled bamboo shoots (43). An increase in the temperature during sterilization could decompose vitamin C, affect nutrition, and cause deterioration of the color and the softness of the structure. As shown in Fig. 4C, the content of vitamin C decreased severely after high-pressure steam sterilization, and only approximately 30% remained. Microwave sterilization was better than high-pressure steam, as 85% of the vitamin C remained. HHP had little effect on vitamin C, as less than 10% was lost in both sample groups. The loss of vitamin C during HHP processing was due to the oxidation reaction; oxygen inside the package could transform vitamin C into dehydroascorbate (DHAA) (44). Vitamin C can also be lost with the extrusion of water. HHP can maintain the nutrients of soft-packed boiled bamboo shoots better than thermal and microwave sterilization.

In conclusion, it was demonstrated that the HHP process could sterilize soft-packed boiled bamboo shoots effectively and could maintain the sensitive nutrition better than thermal or microwave sterilization. Processing at a pressure of 400 MPa for 6 min or 500 MPa for 3 min at room temperature can exterminate all of the

Fig. 4. Quality change of soft-packaged boiled bamboo shoots after different sterilization processes. (A) softness, (B) soluble protein, (C) vitamin C

pathogenic microorganisms and control the total number of colonies at a low level below 100 CFU/g. The sterilization rate of the HHP process was 99.5%. Softness was slightly affected by HHP, and more than 95% and 90% of the soluble protein and vitamin C remained. The improved Weibull model has good accuracy for soft-packed boiled bamboo shoot HHP sterilization ($200 < P < 500$ MPa, $0 < t < 20$ min, room temperature) and could be used to predict the sterilization rate of these products.

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