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ORIGINAL ARTICLE

Shelf life prediction and food safety risk assessment of an innovative whole soybean curd based on predictive models

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Abstract The aim of the present study is to predict the shelf life and evaluate the risk profile of an innovative whole soybean curd (WSC). Two main spoilage strains were isolated from spoiled WSC and identified as *B. subtilis* and *B. cereus*. The origin analysis confirmed that *B. subtilis* and *B. cereus* originated from soybean materials and survived in soybean curd. For microbial contamination analysis, thermotolerant coliforms, *E. coli* and *S. aureus* were not detected in soybean curd. The predicted shelf life of WSC and okara-filtered curd that was stored at 10 °C were 141.95 h (5.91 d) and 206.25 h (8.59 d), respectively. Moreover, the models applied in this study exhibited great fitting goodness and the predicted growth parameters were fail-safe. To conclude, introduction of okara into soybean curd reinforced the initial contamination level but didn't significantly increase the risk profile of WSC.

Keywords Predictive modeling · Whole soybean curd · Spoilage bacteria

Introduction

Soybean curd (tofu) is widely consumed in Asian countries owing to its nutritious and cholesterol-free properties (Pontecorvo and Bourne 1978; Serrazanetti et al. 2013; Zhu et al. 2016). The conventional recipe for soybean curd making involves the soaking of soybean seeds, grinding with additional water, cooking for protein denaturation, filtration of okara (insoluble soybean pulp), renneting with coagulant and pressing for final shaping (Kawaguchi et al. 2018). The soybean curd is then cut, packaged and transferred by cold chains for sale (Liu et al. 2013). It is estimated that 47% of the soy materials remained in okara (Liu 1997), which is usually processed into fodder or directly discarded due to its coarse particle size and adverse effect on sensory, rheological and textural properties of soy products (Aravind et al. 2012). But okara is actually a crucial source of dietary fiber (Li et al. 2012). The intake of dietary fibers has been reported to could lower blood cholesterol and help the food to go through the digestive system (Iwamoto et al. 2005). To optimize the manufacture process of soybean curd and enhance the utilization rate of okara in food raw materials, an innovative whole soybean curd (WSC) which eliminates the filtration step and retains the okara in the

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soybean curd is developed. (Joo et al. 2011; Liu et al. 2013; Zhang et al. 2018).

Conventional okara-filtered curd contains 79–87% of moisture and 6–8.4% of protein (Kovats et al. 1984), which is a favorable ground for the growth of microorganisms. It has been proved that even stored at refrigerated temperature, the shelf life of soybean curd is only a few days (Dotsom et al. 1977). Numerous studies have been carried out to evaluate the microbiological safety of soybean curd so far (Fouad and Hegeman 1993; Gu et al. 2018). Among these studies, lactic acid bacteria, *Pseudomonas* and *Enterobacter* were identified as the main spoilage bacteria (Fouad and Hegeman 1993; Rossi et al. 2016). But the species of spoilage bacteria in soybean curd also varied in soybean species, curd age, storage time (Fouad and Hegeman 1993; Tuitemwong and Fung 1991) as well as the environmental conditions, such as storage temperature, package method, air composition and manufacture process (Angeles and Marth 1971; Cha et al. 2003). Key risk factors associated with the food safety of soybean curd include contamination level of raw ingredients (soybean seeds) and the quality of water (FSANZ 2016a). During the growing and harvesting stages, soybean seeds are easily contaminated by *B. cereus*, which is commonly distributed in soil. Common thermal process among curd making is sufficient enough to kill the vegetative cells of *B. cereus* whereas the spores survive since they are heat-resistant. Hence, the introduction of okara into WSC brings potential challenges to food safety. However, to the best of our knowledge, the studies based on shelf life evaluation and spoilage bacteria identification of WSC are yet to be reported.

Conventional methods (e.g. spoilage test, challenge test) that are used to predict the shelf life of products are laborious and time-consuming. Thus, predictive models are considered as alternative methods (Bruckner et al. 2013). The objective of this study is to apply a capable model to provide an accurate growth prediction of spoilage bacteria and a reliable predicted shelf life of WSC.

Materials and methods

Fabrication of whole soybean curd (WSC)

WSC was fabricated according to the methods of Yasir et al. (2007) and Joo et al. (2011) with little modification. Cleared soybean seeds were ground by a grinder (800-Y, X-Hardware™, Zhejiang, China) at rate of 26,000 rpm for 2 min to make soybean powders. A 100 g of soybean powders was fully mixed with 750 mL of distilled water (25–30 °C) by a magnetic stirrer (MYP11-2, Chijiu™ machinery, Shanghai, China) at rate of 700 rpm for 2 min to obtain soybean powder suspension. The suspension was heated at 95 °C for 5 min to denature the protein. 150 mL of heated soybean powder suspension was loaded in a 250 mL beaker and coagulated with D-Glucono-1, 5-lactone solution (GDL, 0.4%, w/v) and incubated at 55 °C for 30 min to form WSC. The freshly-made WSC was stored in refrigerator (10 °C) for further analysis. Another copy of soybean powder suspension was filtered by a 300-mesh sieve (aseptic) to remove the okara. The filtrate was subsequently processed in the procedure as same as WSC to form soybean curd and set as the control group (namely okara-filtered curd, OFC). To figure out the origins of spoilage bacteria in soybean curd, the containers, coagulation solution and fabrication process were all aseptic.

Isolation and identification of the spoilage bacteria in soybean curd

When the soybean curd had been stored at 37 °C for 24 h, a 5 g of curd sample was taken and transferred into an aseptic sampling bag that was filled with 45 mL of sterile saline (0.85%, w/v, NaCl). The mixture was homogenized by a beating homogenizer (HX-4, Huxi Scientific™, Shanghai, China) for 1 min. The serially diluted mixture was poured into melted trypticase soy agar (TSA) medium. After incubation at 37 °C for 48 h, colonies different in morphology on TSA medium were separated and purified for species identification (Lee et al. 2017).

Genomic DNA was extracted using a DNA extraction kit (DP-302, Tiangen® Biochemical, Beijing, China), amplified for 16S rDNA gene and sequenced by polymerase chain reaction (PCR) using universal primers: 27F (5′-AGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCRs were performed in a PCR system (2720 thermal cycler, Applied Biosystems®, CA, USA) under the following procedures: 5 min at 94 °C; 30 cycles of 10 s at 98 °C, 15 s at 55 °C, 15 s at 72 °C; 5 min at 72 °C and held at 4 °C. Sequences of 16 S rDNA were determined using a sequencer (3730XL, Applied Biosystems®, CA, USA). To identify the spoilage bacteria, the sequencing results were analyzed by sequences alignment on EzBioCloud system (https://www.ezbiocloud.net).

Enumeration of total aerobic bacteria, *Escherichia coli*, thermotolerant coliforms, *Staphylococcus aureus* and *Bacillus cereus*

A 5 g of curd sample was fully mixed with 45 mL of sterile saline (0.85%, w/v, NaCl) and homogenized by a beating homogenizer (HX-4, Huxi Scientific™, Shanghai, China) for 1 min. The mixture was serially diluted and spread onto the following selective media. The total aerobic bacteria were counted by an aerobic count medium (AEB522870,

Huayueruike scientific ™, China). Two film plates (TM BE213 and BL210, Oasis Biotech™, China) were applied for numeration of thermotolerant coliforms and *Bacillus cereus*. *Staphylococcus aureus* was counted by a staph express count plate (Petrifilm TM 6493 and 6491, 3 M®, USA).

Bacillus cereus was identified as per the method of ISO (2004). Mannitol egg yolk polymyxin agar and sheep blood agar (Hangwei ™, Hangzhou, China) were applied as selective medium. For *Bacillus subtilis*, series of diluted curd samples were poured into melted nutrient agar culture medium (NA) and incubated at 30 °C for 24 h to 48 h. Genomic DNA was extracted (DNA extraction kit, DP-302, Tiangen® Biochemical, Beijing, China) and species were confirmed by polymerase chain reaction (upstream primer: 5′-GCGGAATCATCCGTATTGGGGCAGA-3′, downstream primers: 5′-AACCTCGCGGGCTTTCTCGC-CAA-3′). Specific amplified bands appear in 137 bp.

Growth modeling of spoilage bacteria in soybean curd

The freshly-made soybean curds were stored at 15, 20, 25 and 30 °C, respectively. During the storing process, 5 g of the curd sample was taken and transferred into TSA medium for colony counting. The modified Gompertz model (Eq. 1) was applied as the primary model to fit the counting colonies of spoilage bacteria.

$$N_t = N_0 + C \times \exp\left\{-\exp\left[\frac{2.718\mu_{\max}}{C} \times (LT - t) + 1\right]\right\} \quad (1)$$

where N_t (log CFU/g) is the log viable count of bacteria at time t (h); N_0 is the log viable count of the initial bacterial counts (log CFU/g); C is the difference between the initial (N_0) and maximum (N_{max}) bacterial counts (log CFU/g), $C = N_{max} - N_0$; μ_{max} is the maximum relative growth rate at $t = M$, of which M is the time when the bacterial counts reaches maximum (h^{-1}); LT is the lag time (h).

To describe the influence of temperature on the lag time and maximum growth rate, exponential decay equation (Eq. 2, Lee et al. 2017) and square root equation (Eq. 3, Zhou et al. 2009) were selected as the secondary model.

$$LT = a_{LT} \times \exp(-b_{LT} \times T) \quad (2)$$

where LT is the lag time (h); a_{LT} and b_{LT} are regression constants; T is the storage temperature (°C).

$$\sqrt{\mu_{\max}} = b \times (T - T_{\min}) \quad (3)$$

where μ_{max} is the maximum relative growth rate (h^{-1}); b is the regression constant; T is the storage temperature (°C); T_{min} is the minimum temperature for microbial growth (°C).

Evaluation of the fitness of models

The fitting goodness of the primary models was evaluated by parameter correlation coefficients (R^2), accuracy factor (A_f, Eq. 4), bias factor (B_f, Eq. 5) and mean-square-error (RMSE, Eq. 6, Ross 1996).

$$A_f = 10^{\frac{\sum \left|\log\left(\frac{\text{predicted value}}{\text{observed value}}\right)\right|}{n}} \quad (4)$$

$$B_f = 10^{\frac{\sum \log\left(\frac{\text{predicted value}}{\text{observed value}}\right)}{n}} \quad (5)$$

$$RMSE = \sqrt{\frac{1}{n}\sum (\text{predicted} - \text{observed})^2} \quad (6)$$

The acceptable prediction zone (APZ) was calculated to validate the secondary models. The relative error (RE) below zero was considered fail-safe while RE above zero was considered fail-dangerous. The range of APZ was set between RE of − 0.5 to 1 (Oscar 2005).

$$\text{RE for } LT = (\text{predicted} - \text{observed})/\text{predicted} \quad (7)$$

$$\text{RE for } \mu_{\max} = (\text{observed} - \text{predicted})/\text{predicted} \quad (8)$$

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The significant difference of lag time and maximum growth rate among different temperatures were analyzed by Duncan's multiple range test (DMRT, $p < 0.05$) using SPSS 13.0. The significant difference of lag time and maximum growth rate between WSC and OFC stored at same temperature was analyzed by t test ($p < 0.05$). Non-linear regression fitting was carried out on Origin 9.0 (OriginLab Corporation®, USA). Univariate solution was applied to calculate the shelf life of soybean curd (Microsoft Excel® 2013, USA).

Results

Microbial contamination of soybean curd

The prevalence of aerobic bacteria and food-borne pathogens (thermotolerant coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*) in soybean curd was exhibited in Table 1. Obviously, pathogenic bacteria were not detected in all samples, whereas the average values of aerobic bacteria counts in OFC and WSC were 1.62 ± 0.11 log CFU/g and 2.40 ± 0.10 log CFU/g, which presented significant difference ($p < 0.05$).

Table 1 Prevalence of aerobic bacteria and food-borne pathogen in OFC and WSC

Bacteria	Prevalence in OFC	Prevalence in WSC
Aerobic bacteria	1.62 ± 0.11[a]	2.40 ± 0.10
Thermotolerant *coliforms*	N.D.	N.D.
Escherichia coli	N.D.	N.D.
Staphylococcus aureus	N.D.	N.D.
Bacillus cereus	N.D.	N.D.

Each value is expressed as mean ± standard deviation (SD) (n = 3, log CFU/g)

OFC okara-filtered curd; *WSC* whole soybean curd; *N.D.* Not detected

[a]Means significant difference of microbial counts between curd samples by Duncan's multiple range test (DMRT, $p < 0.05$)

Identification and origin analysis of spoilage bacteria in soybean curd

Twelve colonies different in morphology on TSA medium were picked, purified and identified. Two strains (T-1 and T-2) of spoilage bacteria were isolated from OFC and WSC and identified as *Bacillus subtilis subsp. Inaquosorum* (99.93% similarity, 100% completeness) and *Bacillus cereus* (100% similarity, 100% completeness), whose taxonomic results were exhibited in supplementary materials. The prevalence of *Bacillus cereus* and *Bacillus subtilis* in soybean powder, water, soybean powder suspension and soybean curd was exhibited in Table 2. Positive results of *Bacillus cereus* and *Bacillus subtilis* were detected in soybean powder, soybean powder suspension and soybean curd, while negative results were detected in water.

Predictive modeling and validation

The fitted growth curves of spoilage bacteria in soybean curds that were stored at 15, 20, 25 and 30 °C were shown in Fig. 1a, b. The initial microbial populations of WSC and OFC were 3 log CFU/g and 2 log CFU/g, which increased to about 8 log CFU/g after incubation. Table 3 summarized the growth parameters and fitting goodness of spoilage bacteria in soybean curd. Not surprisingly, the lag time of spoilage bacteria in OFC and WSC significantly decreased with increased storage temperature, whereas the maximum growth rate exhibited opposite trends. Additionally, the lag time of OFC was longer than that of WSC at 15, 20, 25 and 30 °C, while no significant difference of maximum growth rate was detected between OFC and WSC at these temperatures. The calculated differences (C value) between initial (N_0) and maximum (N_{max}) microbial population at different temperature were stable in this study (WSC: 4.96–5.22, OFC: 5.96–6.17). From statistical point of view, the regression results (R^2, A_f, B_f and RMSE) agreed with the fitting goodness of the modified Gompertz equation.

The regression curves of growth parameters (lag time and μ_{max}) were shown in Fig. 1c, d. Obviously, the fitted curves were all within the 95% confidence internal. Table 4 exhibited the predictive growth parameters and exponential equations of spoilage bacteria in soybean curds that were incubated at 10 °C and 35 °C. The regression data obtained from the quadratic exponential equation followed a high correlation coefficient ($R^2 > 0.98$) in all test groups. The APZ analyses of growth parameters that were shown in Fig. 1e, f indicated that the relative errors of lag time and μ_{max} were all within the APZ limit.

Shelf life prediction of soybean curd

The predictive growth curves of spoilage bacteria in WSC and OFC that were incubated at 10 °C and 35 °C were shown in Fig. 2. It was worth noting that the shelf life of soybean curd ended when the microbial population reached to 6 log CFU/g (Lee et al. 2017). Univariate solution indicated that the shelf life of WSC that were incubated at 10 °C and 35 °C were 141.95 h (5.91 d) and 3.28 h, of OFC were 206.25 h (at 10 °C) and 5.37 h (at 35 °C).

Discussion

To analyze the sanitation conditions of soybean curd, numerous studies had focused on the identification and growth inhibition of spoilage bacteria, and shelf life extension of soybean curd (Anbarasu and Vijayalakshmi 2010; Kooij and Boer 1985). Rossi et al. (2016) studied the abundance and composition of the microbiota in ready-to-eat fresh tofu. The obtained evidence indicated that appropriate hygienic conditions prevented the contamination of coliforms, whereas the cross contamination

Table 2 Prevalence of *Bacillus cereus* and *Bacillus subtilis* in soybean powder, water, suspension and soybean curd

Bacteria	Soybean powders	Water	Suspension	OFC	WSC
Bacillus cereus	+	−	+	+	+
Bacillus subtilis	+	−	+	+	+

OFC okara-filtered curd; *WSC* whole soybean curd

+ Positive results; − negative results

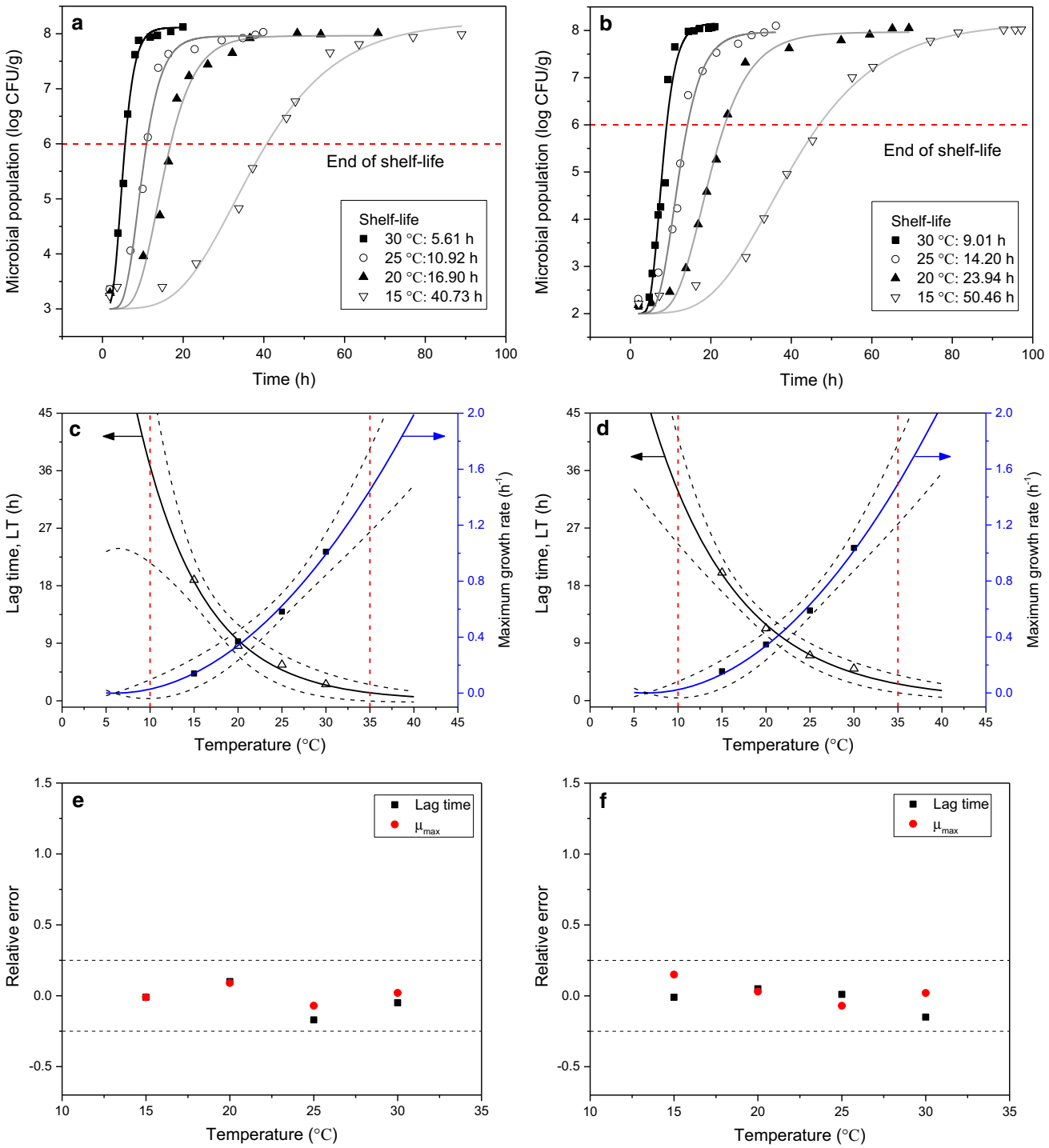

Fig. 1 Fitted growth curves of spoilage bacteria (**a** for WSC and **b** for OFC); Regression curves of growth parameters of spoilage bacteria (**c** for WSC and **d** for OFC); Acceptable prediction zone of growth parameters of spoilage bacteria (E for WSC and F for OFC) (filled square, open circle, filled triangle, open inverted triangle): observed counts; (): fitted curves; (……): 95% CI

introduced *S. macedonicus* and *M. caseolyticus*, which were not commonly associated with soy-based products. In another study, *Listeria monocytogenes* was inoculated into tofu products containing background microflora, whose survival and growth conditions were evaluated. The results confirmed that *L. monocytogenes* strains occupied the dominant population after incubation, thus posing a potential food risk to health (Liu et al. 2010).

The results of above research varied in the types of soybean curd and hygiene condition of the environment.

Table 3 Growth parameters of spoilage bacteria in OFC and WSC that were incubated at 15, 20, 25 and 30 °C

Soybean curd	Temperature (°C)	Lag time (h)	μ_{max} (h^{-1})	C (log CFU/g)	Statistical and validation analysis			
					R^2	A_f	B_f	RMSE
OFC	15	20.01 ± 1.51dA	0.15 ± 0.01aA	6.17	0.99	1.04	1.00	0.48
	20	11.31 ± 0.57cB	0.35 ± 0.02bA	5.96	0.99	1.04	0.98	0.42
	25	7.07 ± 0.75bA	0.59 ± 0.09cA	5.97	0.98	1.06	0.98	0.70
	30	4.99 ± 0.35aB	1.04 ± 0.13dA	6.16	0.97	1.08	0.99	0.96
WSC	15	18.91 ± 2.01dA	0.14 ± 0.01aA	5.22	0.99	1.05	0.98	0.50
	20	8.57 ± 0.91cA	0.37 ± 0.05bA	4.96	0.98	1.05	0.98	0.87
	25	5.63 ± 0.58bA	0.58 ± 0.07cA	4.96	0.98	1.00	1.02	0.85
	30	2.58 ± 0.27aA	1.01 ± 0.10dA	5.11	0.99	1.02	1.00	0.38

Each value is expressed as mean ± standard deviation (SD); Lower case letters (a, b, c, d) mean significance difference of lag time and maximum growth rate among different temperature by Duncan's multiple range test (DMRT, $p < 0.05$); Capital letters (A, B) mean significant difference of lag time and maximum growth rate of spoilage bacteria between OFC and WSC that were incubated at same temperature by t-test ($p < 0.05$)

OFC okara-filtered curd; *WSC* whole soybean curd; μ_{max} maximum growth rate (h^{-1}); *C* the difference between initial (N_0) and final (N_{max}) bacterial numbers: $C = N_{max} - N_0$ (log CFU/g); A_f accuracy factor; B_f bias factor; *RMSE* root mean square error

Table 4 Predictive growth parameters of spoilage bacteria in soybean curds that were incubated at 10 °C and 35 °C

Growth parameters and equations	WSC		OFC	
	10 °C	35 °C	10 °C	35 °C
N_0 (log CFU/g)	3.05	3.11	2.09	2.05
C (log CFU/g)	4.99	4.93	5.97	6.01
μ_{max} (h^{-1})	0.03	1.45	0.02	1.49
LT (h)	36.73	1.25	32.80	2.60
LT equation	$LT = 142.1703 \times \exp(-0.1353T)$, $R^2 = 0.9845$		$LT = 90.3600 \times \exp(-0.1013T)$, $R^2 = 0.9901$	
μ_{max} equation	$\mu_{max} = \{0.0414 \times (T - 5.9076)\}^2$, $R^2 = 0.9895$		$\mu_{max} = \{0.0428 \times (T - 6.4143)\}^2$, $R^2 = 0.9910$	

WSC whole soybean curd; *OFC* okara-filtered curd; N_0 initial bacterial level of soybean curd (observed value, log CFU/g); *C* difference between initial (N_0) and maximum (N_{max}) bacterial numbers: $C = N_{max} - N_0$ (log CFU/g); *LT* lag time (h); μ_{max} maximum growth rate (h^{-1}); *T* temperature (°C)

Few studies had concerned about the microbial evaluation of innovative soybean curd. Therefore, a comparative analysis containing microbial contamination, origin analysis and growth prediction of spoilage bacteria in WSC was carried out in this study, which was aimed to provide more technical references for this innovative soybean products.

Aerobic bacteria were detected in OFC and WSC, albeit in low level. Common filled soybean curd was soaked with water or whey, thus the internal environment of soybean curd was anaerobic and the growth of aerobic bacteria was inhibited. Lee et al. (2017) analyzed the population distribution of aerobic bacteria in 100 commercial tofu in Korea, and only 32% of the samples showed positive detection results of aerobic bacteria. Moreover, 59.4% of the positive samples exhibited a contamination level below 3 log CFU/g, which was in consistent with our results. The counts of aerobic bacteria in WSC was significantly higher ($p < 0.05$) than that of OFC, indicating that the introduction of okara into soybean curd reinforced the initial contamination level because additional sources of substrates were provided.

As the common food-borne pathogens, coliforms were previously detected in soybean curd (Ananchaipattana et al. 2012; Szabo et al. 1989), which indicated that the curd samples had been contaminated by faeces. In this study, food-borne pathogens including thermotolerant coliforms, *E. coli*, *S. aureus* and *B. cereus* were not detected in freshly-made OFC and WSC. Hence, the introduction of okara into soybean curd didn't increase the infection risk of food-borne pathogens when good hygienic conditions were adopted in the manufacture process of soybean curd. Considering that false-negative results may occur when *B.*

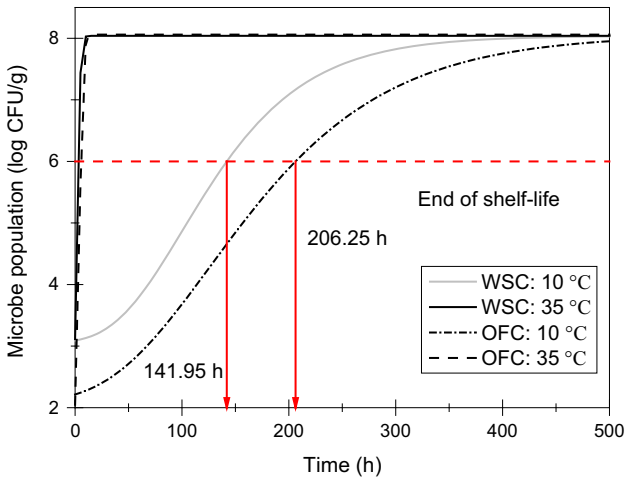

Fig. 2 Predictive growth curves of spoilage bacteria in WSC and OFC that were incubated at 10 °C and 35 °C

cereus was at spore state, 16S rDNA sequencing was applied for bacteria identification. Two main spoilage strains were isolated from soybean curd and identified as *Bacillus subtilis subsp. Inaquosorum* and *Bacillus cereus*, respectively. *B. cereus* is a gram-positive, endospore-forming and facultative anaerobic pathogen, which has been detected in cooked beans and soybean curd (Juneja et al. 2018; Lee et al. 2017). If the environmental conditions were suitable, the spores of *B. cereus* would germinate. This condition usually occurred after the thermal process of soybean curd making (MPI 2016). The ingestion of *B. cereus* cells and heat-stable emetic toxin produced by *B. cereus* in foods will lead to food borne illness (Carlin et al. 2006). *B. subtilis* were widely used in fermented food industry and had gained "generally recognized as safe" (GRAS) status (Yeo et al. 2012). However, some *Bacillus subtilis* groups (e.g. *B. subtilis*, *B. licheniformis* and *B. pumilis*) produced toxin and caused illness as well, but not all *Bacillus subtilis* was associated with illness (FSANZ 2016a).

Spoilage bacteria in soybean curd originate from a wide range of sources including soybean materials, water, packaging materials and so on. In this study, *B. subtilis* and *B. cereus* were detected in soybean powder, soybean powder suspension and final soybean curd but absent in water. Hence, there was a strong possibility that *B. subtilis* and *B. cereus* originated from soybean materials and survived in soybean curd. In the practical production, additional heat treatment was usually applied to sterilize the formed soybean curd. However, due to the presence of *Bacillus* spores, heat treatment might be ineffective. Thus, irradiation sterilization technology could be considered as an alternative.

Application of quantitative mathematical equations for predicting microbial growth is the first step to design the primary models as well as the real-time quality controller (Phua and Davey 2007). The growth curves of spoilage bacteria in soybean curd that were depicted in Fig. 1a, b were built on the observed microbial counts. Obviously, the observed initial microbial counts (N_0) of WSC were about 1.5 times of that of OFC. Therefore, the introduction of okara into soybean curd significantly increased the initial contamination level. As the major environmental factor that affects the growth kinetics of microorganisms in food storage process, temperature played an essential role on the growth conditions of spoilage bacteria and consequently on the quality of products. According to the results in Table 3, the difference of lag time between OFC and WSC might result from the different initial contamination level. The occurrence of lag phase could result from the changes of physicochemical environment, because lag time was dependent on the history and initial biochemical state of the bacterial cells. Once the bacteria grow logarithmically, the growth rate would be changed by temperature almost instantaneously (Baranyi et al. 1995). Therefore, in this sense, μ_{max} was a parameter that described the growth of microorganisms in a particular environment (Sant'Ana et al. 2012). To evaluate the precision of the primary model, accuracy factor (A_f), bias factor (B_f) and root mean square error (RMSE) were calculated based on the microbial counts of each isothermal condition. The accuracy factor varied from 1.00 to 1.08, which meant that compared with the observed values, the prediction varied between 0% and 8%. B_f represented the deviation between predicted and observed values. An average B_f value < 1 for modeling meant underprediction occurred, but a B_f value within a range of 0.90–1.05 is still considered good. RMSE is a measurement accounting for the difference between predicted and observed values. A lower RMSE value showed that more precise experiment data were described (Ross 1996). To conclude, these results suggested that when the soybean curds were stored at a constant temperature (15, 20, 25, and 30 °C), the models built herein exhibited great fitting goodness.

Regression curves of growth parameters of spoilage bacteria in soybean curd that were shown in Fig. 1c, d were all within the 95% CI. APZ analysis shown in Fig. 1e, f was applied to evaluate the acceptance of the prediction. Obviously, all of the relative errors were within the acceptable prediction zone. Thus, the predictions of growth parameters were fail-safe.

Reasonable extrapolation of model predictions to conditions that were not experimented in the model was an effective way to save time and money for food innovation. To obtain the shelf life of soybean curd that was stored at 10 °C (refrigerated temperature) and 35 °C, the lag time and μ_{max} of soybean curd were calculated by the predictive exponential equations shown in Table 4. The initial

numbers of microbial counts were determined. The maximum bacterial counts (N_{max}) was calculated by averaging the bacterial counts that were obtained at other isothermal conditions (15, 20, 25 and 30 °C). As expected, when stored at refrigerated temperature (10 °C), the shelf life of WSC was 5.91 d, which was 31.17% shorter than OFC. However, when the storage temperature increased to 35 °C, the shelf life of WSC decreased 38.92% in comparison with OFC. Hence, the introduction of okara shortened the shelf life of soybean curd, whereas the shortened shelf life was still acceptable.

Consumption risk assessment of soybean curd involved the identification of pathogenic microorganisms and methodical description of the system. As a moderate hazard, *B. cereus* usually causes illness of short duration and has no sequelae. All population might be susceptible to *B. cereus* poisoning (FSANZ 2016b). Agata et al. (2002) confirmed that the number of *B. cereus* was required to reach 10^6 to produce emetic toxin in foods. FSANZ (2016b) categorized satisfactory evaluation for foods if *B. cereus* level was below 10^2 CFU/g. However, it was deemed that potential hazard needed to be evaluated if *B. cereus* level was over 10^5 CFU/g.

According to the compendium of microbiological criteria for food in Australia (FSANZ 2016b), *B. cereus* was considered as the main pathogen in soybean curd. Other species of *Bacillus* associated with food-borne illness are from the *Bacillus subtilis* group (including *B. subtilis*, *B. licheniformis* and *B. pumilis*). Symptoms of illness and causative factors caused by *Bacillus subtilis* group were similar to *B. cereus* (FSANZ 2016a). FAO/WHO expert consultations (2004, 2006) categorized *Bacillus cereus* as "Microorganisms for which causality with illness is less plausible or not yet demonstrated" (Category C) and it was generally acceptable that low levels ($< 10^2$ CFU/g) of these *Bacillus* groups might exist in powdered infant formula products and should be monitored by the manufacturers appropriately.

Conclusion

The present study firstly predicted the shelf life and evaluated the food safety risk of an innovative whole soybean curd. The results suggested that thermotolerant coliforms, *E. coli* and *S. aureus* were not detected in OFC and WSC. Two dominant spoilage strains were isolated from spoiled OFC and WSC and identified as *Bacillus subtilis subsp. Inaquosorum* and *Bacillus cereus* using 16S rDNA sequencing technology. Moreover, the origin analysis confirmed that spoilage bacteria originated from soybean materials and survived in soybean curd. To estimate the shelf life of WSC, modified Gompertz equation was applied as the primary model. The maximum growth rate and lag time of spoilage bacteria derived from primary model were fitted to exponential decay equation and square root equation. The initial contamination level of WSC was one order of magnitude higher than that of OFC. The predicted shelf life of WSC and OFC stored at 10 °C were 141.95 h (5.91 d) and 206.25 h (8.59 d), respectively. Statistical and validation analyses supported the predictive purpose. To conclude, the introduction of okara into soybean curd reinforced the initial level of contamination but didn't significantly increase the risk profile of WSC.

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References

Agata N, Ohta M, Yokoma K (2002) Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. Int J Food Microbiol 73:23–27. https://doi.org/10.1016/S0168-1605(01)00692-4

Ananchaipattana C, Hosotani Y, Kawasaki S, Pongsawat S, Latiful BM, Isobe S et al (2012) Bacterial contamination of soybean curd (tofu) sold in Thailand. Food Sci Technol Res 18:843–848. https://doi.org/10.3136/fstr.18.843

Anbarasu K, Vijayalakshmi G (2010) Improved shelf life of protein-rich tofu using Ocimum sanctum (tulsi) extracts to benefit Indian rural population. J Food Sci 72:300–305. https://doi.org/10.1111/j.1750-3841.2007.00487.x

Angeles AG, Marth EH (1971) Growth and activity of lactic-acid bacteria in soymilk: I. Growth and acid production. J Milk Food Technol 34:30–36. https://doi.org/10.4315/0022-2747-34.1.30

Aravind N, Sissons MJ, Fellows CM, Blazek J, Gilbert EP (2012) Effect of inulin soluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti. Food Chem 132:993–1002. https://doi.org/10.1016/j.foodchem.2011.11.085

Baranyi J, Robinson TP, Kaloti A, Mackey BM (1995) Predicting growth of *Brochothrix thermosphacta* at changing temperature. Int J Food Microbiol 27:61–75. https://doi.org/10.1016/0168-1605(94)00154-X

Bruckner S, Albrecht A, Petersen B, Kreyenschmidt J (2013) A predictive shelf life model as a tool for the improvement of quality management in pork and poultry chains. Food Control 29:451–460. https://doi.org/10.1016/j.foodcont.2012.05.048

Carlin F, Fricker M, Pielaat A, Heisterkamp S, Shaheen R, Salonen MS, Svensson B et al (2006) Emetic toxin-producing strains of *Bacillus cereus* show distinct characteristics within the *Bacillus cereus* group. Int J Food Microbiol 109:132–138. https://doi.org/10.1016/j.ijfoodmicro.2006.01.022

Cha DS, Chen J, Park HJ, Chinnan MS (2003) Inhibition of *Listeria monocytogenes* in tofu by use of a polyethylene film coated with a cellulosic solution containing nisin. Int J Food Sci Tech 38:499–503. https://doi.org/10.1046/j.1365-2621.2003.00684.x

Dotsom CR, Frank HA, Cavaletto CG (1977) Indirect methods as criteria of 329 spoilage in tofu (soybean curd). J Food Sci 42:273–274. https://doi.org/10.1111/j.1365-2621.1977.tb01270.x

FAO/WHO (2004) *Enterobacter sakazakii* and microorganisms in powdered infant formula: Meeting Report. https://www.who.int/foodsafety/publications/mra6-enterobacter-sakazakii/en/

FAO/WHO (2006) *Enterobacter sakazakii* and Salmonella in powdered infant formula: Meeting Report. https://www.who.int/foodsafety/publications/micro/mra10/en/

Food Standards Australia New Zealand (FSANZ) (2016) Advice on compendium of microbiological criteria for food, *Bacillus cereus* and other *Bacillus* spp. http://www.foodstandards.gov.au/publications/pages/compendium-of-microbiological-criteria-for-food.aspx. Accessed Oct 2016

Food Standards Australia New Zealand (FSANZ) (2016) Advice on compendium of microbiological criteria for food, Bean curd and *Bacillus cereus*. http://www.foodstandards.gov.au/consumer/importedfoods/Pages/FSANZ-advice-on-imported-food.aspx. Accessed Nov 2016

Fouad KE, Hegeman GD (1993) Microbial spoilage of tofu (soybean curd). J Food Protect 56:157–164. https://doi.org/10.4315/0362-028X-56.2.157

Gu J, Liu T, Sadiq FA, Yang H, Yuan L, Zhang G, He G (2018) Biogenic amines content and assessment of bacterial and fungal diversity in stinky tofu-A traditional fermented soy curd. LWT-Food Sci Technol 88:26–34. https://doi.org/10.1016/j.lwt.2017.08.085

ISO (2004) Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of presumptive *Bacillus cereus*-Colony-count technique at 30 degrees C. https://www.iso.org/standard/38219.html

Iwamoto S, Nakagaito AN, Yano H, Nogi M (2005) Optically transparent composites reinforced with plant fiber-based nanofibers. Appl Phys A-Mater Sci Process 81:1109–1112. https://doi.org/10.1007/s00339-005-3316-z

Joo SI, Kim JE, Lee SP (2011) Physicochemical properties of whole soybean curd prepared by microbial transglutaminase. Food Sci Biotechnol 20:437–444. https://doi.org/10.1007/s10068-011-0061-2

Juneja VK, Mishra A, Pradhan AK (2018) Dynamic predictive model for growth of *Bacillus cereus* from spores in cooked beans. J Food Protect 81:308–315. https://doi.org/10.4315/0362-028X.JFP-17-391

Kawaguchi T, Kita R, Shinyashiki N, Yagihara S, Fukuzaki M (2018) Physical properties of tofu gel probed by water translational/rotational dynamics. Food Hydrocolloid 77:474–481. https://doi.org/10.1016/j.foodhyd.2017.10.025

Kooij JAV, Boer ED (1985) A survey of the microbiological quality of commercial tofu in the Netherlands. Int J Food Microbiol 2:349–354. https://doi.org/10.1016/0168-1605(85)90025-X

Kovats SK, Doyle MP, Tanaka N (1984) Evaluation of the microbiological safety of tofu. J Food Protect 47:618–622. https://doi.org/10.4315/0362-028X-47.8.618

Lee DY, Kwon KH, Chai C, Oh SW (2017) Microbial contamination of tofu in Korea and growth characteristics of *Bacillus cereus* isolates in Tofu. LWT-Food Sci Technol 78:63–69. https://doi.org/10.1016/j.lwt.2016.11.081

Li B, Qiao MY, Lu F (2012) Composition, nutrition, and utilization of Okara (Soybean Residue). Food Rev Int 28:231–252. https://doi.org/10.1080/87559129.2011.595023

Liu K (1997) Soybeans: chemistry, technology and utilization. Chapman and Hall, New York

Liu JG, Lin TS, Lin WY (2010) Evaluating the growth of Listeria monocytogenes that has been inoculated into tofu containing background microflora. Food Control 21:1764–1768. https://doi.org/10.1016/j.foodcont.2010.09.001

Liu HH, Chien JT, Kuo MI (2013) Ultra high pressure homogenized soy flour for tofu making. Food Hydrocolloid 32:278–285. https://doi.org/10.1016/j.foodhyd.2013.01.005

Ministry for Primary Industries (MPI) (2016). Custom food control plan. Ministry for Primary Industries, New Zealand. https://www.foodsafety.govt.nz/elibrary/industry/food-control-food-fcp/food-control-plan-version-5.pdf. Accessed 11 Aug 2016

Oscar TE (2005) Validation of lag time and growth rate models for *Salmonella Typhimurium*: acceptable prediction zone method. J Food Sci 70:129–137. https://doi.org/10.1111/j.1365-2621.2005.tb07103.x

Phua STG, Davey KR (2007) Predictive modelling of high pressure (<=700 MPa)-cold pasteurisation (<=25°C) of *Escherichia coli*, *Yersinia enterocolitica* and *Listeria monocytogenes* in three liquid foods. Chem Eng Process 46:458–464. https://doi.org/10.1016/j.cep.2006.03.020

Pontecorvo AJ, Bourne MC (1978) Simple methods for extending the shelf life of soy curd (tofu) in tropical areas. J Food Sci 43:969–972. https://doi.org/10.1111/j.1365-2621.1978.tb02464.x

Ross T (1996) Indices for performance evaluation of predictive models in food microbiology. J Appl Microbiol 81:501–508. https://doi.org/10.1111/j.1365-2672.1996.tb03539.x

Rossi F, Felis GE, Martinelli A, Calcavecchia B, Torriani S (2016) Microbiological characteristics of fresh tofu produced in small industrial scale and identification of specific spoiling microorganisms (SSO). LWT-Food Sci Technol 70:280–285. https://doi.org/10.1016/j.lwt.2016.02.057

Sant'Ana AS, Franco BD, Schaffner DW (2012) Modeling the growth rate and lag time of different strains of *Salmonella enterica* and *Listeria monocytogenes* in ready-to-eat lettuce. Food Microbiol 30:267–273. https://doi.org/10.1016/j.fm.2011.11.003

Serrazanetti DI, Ndagijimana M, Miserocchi C, Perillo L, Guerzoni ME (2013) Fermented tofu: enhancement of keeping quality and sensorial properties. Food Control 34:336–346. https://doi.org/10.1016/j.foodcont.2013.04.047

Szabo RA, Jarvis GA, Weiss KF, Rayman K, Lachapelle G, Jean A (1989) Microbiological quality of tofu and related products in Canada. J Food Protect 52:727–730. https://doi.org/10.4315/0362-028X-52.10.727

Tuitemwong K, Fung D (1991) Microbiological study of tofu. J Food Protect 54:212–216. https://doi.org/10.4315/0362-028X-54.3.212

Yasir SBM, Sutton KH, Newberry MP, Andrews NR, Gerrard JA (2007) The impact of transglutaminase on soy proteins and tofu texture. Food Chem 104:1491–1501. https://doi.org/10.1016/j.foodchem.2007.02.026

Yeo IC, Lee NK, Hahm YT (2012) Genome sequencing of *Bacillus subtilis* SC-8, antagonistic to the *Bacillus cereus* group, isolated from traditional Korean fermented-soybean food. J Bacteriol 194:536–537. https://doi.org/10.1128/JB.06442-11

Zhang Q, Wang CZ, Li BK, Li L, Lin DR, Chen H, Liu YW et al (2018) Research progress in tofu processing: from raw materials to processing conditions. Crit Rev Food Sci 58:1448–1467. https://doi.org/10.1080/10408398.2016.1263823

Zhou K, Fu P, Li PL (2009) Predictive modeling and validation of growth at different temperatures of *Brochothrix thermosphacta*. J Food Safety 29:460–473. https://doi.org/10.1111/j.1745-4565.2009.00169.x

Zhu QM, Wu FF, Saito M, Tatsumi T, Yin L (2016) Effect of magnesium salt concentration in water-in-oil emulsions on the physical properties and microstructure of tofu. Food Chem 201:197–204. https://doi.org/10.1016/j.foodchem.2016.01.065