Sequential Quadratic Programming for Development of a New Probiotic Dairy Tofu with Glucono-δ-Lactone

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ABSTRACT: The purpose of this research was to evaluate the effects of various concentrations of glucono-δ-lactone (GDL) and skim milk powder, as well as the addition of prebiotics, on the rheology and probiotic viabilities of dairy tofu. Additionally, modern optimization techniques were applied to attempt to determine the optimal processing conditions and growth rate for the selected probiotics (*Lactobacillus. acidophilus, L. casei, Bifidobacteria bifidum*, and *B. longum*). There were 2 stages in this research to accomplish the goal. The 1st stage was to derive surface models using response surface methodology (RSM); the 2nd stage performed optimization on the models using sequential quadratic programming (SQP) techniques. The results were demonstrated to be effective. The most favorable production conditions of dairy tofu were 1% GDL, 0% peptides, 3% isomaltooligosaccharides (IMO), and 18% milk, as confirmed by subsequent verification experiments. Analysis of the sensory evaluation results revealed no significant difference between the probiotic dairy tofu and the GDL analog in terms of texture and appearance ($P > 0.05$). The viable numbers of probiotics were well above the recommended limit of 10^6 CFU/g for the probiotic dairy tofu throughout the tested storage period.

Keywords: sequential quadratic programming, glucono-δ-lactone, probiotics, dairy tofu

Introduction

In recent years, there has been a worldwide increase in the consumption of fermented milk, especially probiotic products. Probiotics are defined as "the viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance" (Kaur and others 2002). The addition of probiotic bacteria to fermented milk products is made not only as a consequence of certain claimed health-promoting effects of their presence in intestinal tract of consumers, but also due to the expanding variety of products that can be formulated with probiotics bacteria. It seems reasonable to suggest that developing new probiotics-based dairy products may provide added consumer variety, and enhance the robustness of the dairy industry by stimulating demand (Liu and others 2002).

Glucono-δ-lactone (GDL) is allowed for use in human food as a coagulant and a pH control agent. Compared with other food acids, GDL provides a gradual, progressive, and continuous decrease of pH to equilibrium due to its slow hydrolysis to gluconic acid. Accordingly, it is used as a slow release acidulant. During its hydrolysis, its initial sweet taste becomes only slightly acidic, making the final flavor of an aqueous solution much less tart than that of other acidulants (Schwertfeger and Buchheim 1999). Historically, tofu coagulants were either a combination of magnesium chloride and magnesium sulfate or mined calcium sulfate. GDL tofu is made by coagulation of soymilk with GDL to produce a soy protein gel, which traps water, lipids, and other constituents in the matrix (Kim and Han 2002).

The concept of probiotic dairy tofu was adapted from GDL tofu and yogurt. This new product was made by addition of prebiotics in milk and coagulation of milk mixed with GDL along with probiotics to form a silken smooth milk gel. Prebiotics are non-digestible food ingredients that beneficially affect that host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Kaur and others 2002). Bielecka and others (2002) confirmed the appropriate selection of probiotics as well as prebiotics and demonstrated their higher effectiveness as compared with probiotics. The probiotic dairy tofu, containing both probiotics and prebiotics, offers the texture of tofu and the flavor of yogurt, as well as related health benefits.

There have been several reports exploring the rheological properties of GDL-formed acid milk gels (Arshad and others 1993; Lucey and others 1998; Lucey and Singh 1998) and bacterial fermentation (Biliaderis and others 1992; Lucey and others 1998), however, none have studied the combination of GDL with probiotics and prebiotics to create a new dairy product.

To develop a good quality dairy tofu and elucidate the effects of the different ingredients in terms of the chemical, physical, and microbial properties of this product, response surface models were developed to describe the combined effect of the factors, and modern optimization techniques were applied to attain optimal conditions for the manufacturing process.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. It usually consists of 3 stages (Myers and Montgomery 1995): (1) experimental design, (2) response surface modeling through regression, and (3) optimization. The main advantage of RSM is a reduction in the number of experimental trials needed to evaluate multiple parameters and determine their interactions (Porretta and others 1995; Lee and others 2000). Experimental data were utilized to build mathematical models using the regression method. Once an appropriate approximating model has been derived, it can be analyzed using various optimization techniques to determine the optimum conditions for the process. RSM has been successfully applied to determine the optimum production conditions for the dairy product, Kou Woan Lao (Weng and others 2001) and for development

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of new edible gels (Chen and Lin 2002).

The objective of the present study was the creation of a new dairy product, probiotics dairy tofu, by blending skim milk with GDL and inoculating it with selected probiotics. The purpose of this research was, therefore, to evaluate the effects of GDL concentration and skim milk powder, as well as the addition of prebiotics, on the rheology and probiotic viabilities of this new dairy tofu. Additionally, response surface modeling and modern optimization techniques were applied to attempt to determine the optimal processing conditions and growth rate for the selected probiotics.

Materials and Methods

Preparation of probiotic dairy tofu

According to the results of our preliminary tests, insignificant treatments were eliminated and 4 significant treatments, concentrations of skin milk powder, peptides, isomaltooligosaccharides (IMO), and GDL, were selected. The preliminary tests also revealed that GDL along with probiotics could build a silken milk gel and improve the flavor of the acid milk gel, and that GDL levels between 0.3% and 1.0% provided a balance between coagulation and flavor of the samples. Additionally, the results showed that dairy tofu with pH of 4.4 to 4.5 yielded better flavor and texture. As a consequence, the samples were prepared using 12% to 18% (w/w) skim milk powder (Anchor Foods, Manurewa, New Zealand). Reconstituted skim milk in deionized water (pH 6.7, protein 4.0% to 5.9%) was mixed with prebiotics (peptides 0.0% to 1.0% and IMO 0.0% to 3.0%; Cheng-Fung Co., Taipei, Taiwan), and then heated at 85 °C for 30 min. After cooling to 37 °C, the mixtures were blended with 0.3% to 1% GDL and inoculated immediately with *Lactobacillus acidophilus*, *L. casei*, *Bifidobacteria bifidum*, and *B. longum*. When the samples reached to pH values of 4.4 to 4.5 (fermented approximately 7 to 8 h), the incubation was halted by cooling down to 4 °C, and the samples were stored at this temperature for 4 wk.

Culture and medium performance

Pure lyophilized cultures of *B. longum* (CCRC 14605), *L. casei* subsp. *rhamnosus* (CCRC 12321), *B. bifidum* (CCRC 11844), and *L. acidophilus* (CCRC 14079) were purchased from the Culture Collection and Research Center, Hsinchu, Taiwan, R.O.C. Lactobacilli MRS (deMan, Rogosa, and Sharp) and lithium propionate MRS agar (LP-MRS) were used as the selective media for *Lactobacillus* spp. and *Bifidobacteria* spp., respectively (Lapierre and others 1992).

Determination of probiotic growth rate

To determine the probiotic viability, the *Lactobacillus* spp. and *Bifidobacteria* spp. populations and growth rates were measured. The suitability of the media was tested by plating decimal dilutions of the probiotic cultures. Thus, a 1-g sample of each pure lyophilized culture was decimally diluted into sterile peptone water (0.1%), and then 0.1-mL aliquot dilutions were plated onto the different media, in triplicate. Plates of MRS agar were incubated aerobically for 72 h at 37 °C to inhibit bifidobacteria. Plates of LP-MRS agar (GasPak System; Oxoid Unipath Ltd., Basingstoke, Hampshire, England) were incubated anaerobically (72 h at 37 °C) for enumerating the bifidobacteria. The population, in colony-forming units (CFU), and the characteristics of the colonies were recorded for each medium.

The specific growth rate (*GR*) corresponding to each culture was calculated using the following equation:

$$GR = \frac{[\log(CFU_2) - \log(CFU_1)]}{t_2 - t_1} \quad (1)$$

where CFU_1 and CFU_2 are the *CFU* at times t_1 (fermentation for 0 h) and t_2 (fermentation for 7 to 8 h).

Determination of hardness

The hardness of the samples was determined by testing 5 replicate samples on a TA-XT2i/5 Texture Analyser (Stable Micro Systems, scarsdale, N.Y., U.S.A.) fitted with a 5-kg load cell. The gels were formed in a container (50 mm dia, 65 mm height) with 80 mL milk mix and tested using a cylinder probe with a flat-ended head of 20-mm dia at a fixed rate of 20 mm/min. The probe traveled 80% depth into the samples. Gel hardness was expressed as the force (g) at the maximum peak of the force-time curve.

Response surface modeling

Before commencement of experiments, experimental design was performed. The Box-Behnkin Design (BBD; Box and Behnkin 1960) is a three-level design based on the construction of a balanced incomplete block design. The BBD is an efficient option for fitting response surfaces using 3 evenly spaced levels (Myers and Montgomery 1995). It was assumed that the hardness and probiotic viabilities of the probiotic dairy tofu are affected by 4 independent variables. A four-variable BBD with 5 replicates at the center point was selected to build the response surface models. The coded and uncoded variables and their respective levels are shown in Table 1.

To conduct the response surface modeling, the regression method was applied to the experimental results to build mathematical models. The models were then formulated as an objective function in an optimization problem, with optimization techniques used to determine maximum probiotic viability and product hardness. The RSM procedure of the Design-Expert® software package (Stat-Ease Inc., Minneapolis, Minn., U.S.A.) was used to fit the experimental data to polynomial equations in the order 1 through 3, according to the following linear relationship:

$$Y_i = f_i(X_1, X_2, X_3, X_4) + \varepsilon_i \; i = 1,2,3 \quad (2)$$

where Y_1, Y_2, Y_3 are the *Lactobacillus* spp. and *Bifidobacteria* spp. growth rates and the hardness of the dairy tofu, respectively; f_1, f_2, f_3 represent the modeled response surfaces; and X_1, X_2, X_3, X_4, defined as natural variables, are the concentrations of GDL, peptides, IMO, and milk, respectively. The errors for the 3 models are ε_1, ε_2, and ε_3. With RSM, it is convenient to transform the natural variables to the coded analogs, χ_1, χ_2, χ_3, χ_4, which are defined as dimensionless, with zero mean values and the same spreads or standard deviations:

$$Y_i = f_i(\chi_1, \chi_2, \chi_3, \chi_4) + \varepsilon_i \; i = 1,2,3 \quad (3)$$

Optimization of response surface models and model verification

Development of objective function. All measured variables were included in the formulation of the objective function that must be defined for an optimization problem. To search for a solution that maximizes multiple responses, a composite function (CF) was defined as follows:

$$CF = (f_1 \times f_2 \times f_3)^{1/3} \quad (4)$$

The composite function combines 3 responses into 1 single function whose maximum can then be sought by optimization techniques. Each response contributes equally to the composite function.

Optimization by sequential programming. An SQP procedure

Table 1—Process variables and their levels in 4 variables: 3 levels of response surface design

Independent variable	Symbol	Level	
		Coded	Uncoded
GDL concentration (%)	X_1	−1	0.30
		0	0.65
		+1	1.00
Peptides concentration (%)	X_2	−1	0.00
		0	0.75
		+1	1.50
IMO concentration (%)	X_3	−1	0.00
		0	1.50
		+1	3.00
Skim milk concentration (%)	X_4	−1	12.00
		0	15.00
		+1	18.00

implemented in the MATLAB software (The Math Works Inc., Natick, Mass., U.S.A.) was employed to optimize probiotic growth rates and the hardness of the probiotic dairy tofu, with RSM formulated as polynomial functions of 4 independent variables bounded by preset upper and lower limits. The basic SQP technique can be expressed in terms of the following steps (Reklaintis and others 1983; Chen 2003): (1) Set up and solve a quadratic programming subproblem, yielding a search direction. (2) Test for convergence, stop if the process converges. (3) Step forward to a new point along the search direction. (4) Update the Hessian matrix in quadratic programming and go to step 1.

To search for the global optimum, the concept of a multi-start global optimization procedure (Snyman and Fatti 1987) was combined with the SQP method as has been described (Reklaintis and others 1983). Let F^* denote the global maximum, and r be the number of sample points falling within the region of convergence of the current overall maximum F after n points have been sampled. Then, under statistically noninformative prior distribution, the probability that F is equal to F^* satisfies the following relationship:

$$\Pr[F = F^*] \geq q(n, r) = 1 - [(n+1)!(2n-r)!]/[(2n+1)!(n-r)!] \quad (5)$$

In this study, a very high probability (>0.9999) was set in Eq. 5 to ensure that the global optimum would be attained.

Model verification. After optimal processing conditions were determined using SQP, experiments based on these conditions were performed and repeated 3 times. The results were then analyzed using ANOVA from the SAS software package (SAS Inst. Inc., Cary, N.C., U.S.A.) with Duncan's multiple range test for significance used to detect differences between the predicted and observed values.

Scanning electron microscopy

Preparation of samples. Probiotic dairy tofu, whose preparation method has been described previously, was produced according to the formula of the optimized results. The final pH and protein content was 4.4% to 4.5% and 5.72%, respectively.

GDL tofu was prepared by a modification of the method proposed by Kim and Han (2002). Soybeans were washed and soaked in water at room temperature for 12 h, and then the soaked beans were drained and blended for 10 min (Blender A76, Moulinex, Paris, France) with water to give a water-dry beans ratio of 10:1 (weight basis). The mash was cooked for 15 min at boiling temperature with occasional stirring, after which the hot mash was filtered through double layers of cheesecloth and the soymilk was cooled to about 80 °C. Finally, tofu samples were prepared by mixing 2% GDL. The final pH of tofu was 5.7 and protein content was 5.86%.

Plain yogurt was prepared using 18% (w/w) skim milk powder. Reconstituted skim milk in deionized water (pH 6.7) was heated to 85 °C for 30 min. After cooling to 37 °C, the sample was inoculated with *L. delbruckii* spp. *bulgaricus* and *Streptococcus thermophilus* at a concentration of 1%, and then incubated at 37 °C until the sample reached a pH value of 4.4 to 4.5. The protein content of the final product was 5.74%.

GDL acidified milk gel was prepared using a mixture of 18% (w/w) skim milk powder (Anchor Foods) and GDL. Reconstituted skim milk in deionized water was heated to 85 °C for 30 min, and then mixed with 1% GDL at 37 °C to obtain a final pH value of 4.5. The sample was then cooled to 4 °C. The protein content of the product was 5.81%.

Microstructure of samples. The microstructures of the GDL tofu, plain yogurt, GDL-acidified milk gel, and probiotic dairy tofu were observed by scanning electron microscope (SEM) according to the method of Lin and others (1999). The following provides a brief summary of the method. Pieces of samples were fixed in 30 g/L glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) at 25 °C for 4 h. Then, the samples were washed with 3 changes of buffer and post-fixed with 10 g/L osmium tetroxide in the same buffer at 25 °C for 1 h. After washing in distilled water, the samples were dehydrated in an ethanol series: 15%, 30%, 50%, and 70% for 10 min each; 85% and 95% for 15 min each and 100% for 1 h. The resulting specimens were critical-point dried with CO_2 using a Critical Point Dryer Samdri-PVT-3B (Tousimis, Rockville, Md., U.S.A.). Eventually, the samples were fixed in stubs on a double-faced metallic tape and covered with a fine layer of gold (Ion Coater JJFC1100E; JEOL Ltd., Tokyo, Japan) while applying a current of 40 mA, and observed using an SEM (JSM-6300, JEOL Ltd.).

Sensory evaluation

Trained panelists, composed of 4 adult males and 5 females who were familiar with yogurt and tofu, were selected and trained according to the guidelines in ISO 8586-1 (1993). Parameters evaluated were appearance/color, body/texture, and aroma/taste on a 9-point hedonic scale (1 = extremely poor to 9 = excellent). Samples of GDL tofu, plain yogurt, and probiotic dairy tofu were presented to the judges in individual plastic bottles placed on cooled metal blocks to keep gel temperatures low and uniform during testing (1-d-old gels at around 4 to 5 °C). Gels were randomly presented to the panelists during each session and evaluated on 3 separate occasions. Each panelist evaluated the 4 gels 3 times (once per session).

Results and Discussion

Response surface modeling

The results for the probiotic viability and hardness of the probiotic dairy tofu are presented in Table 2. The present work has used RSM to develop a prediction model for establishing the processing conditions for probiotic dairy tofu. The responses, as linear, quadratic, and cubic functions of the variables, were tested for adequacy and fitness using analysis of variance (ANOVA). Model analysis, lack-of-fit test, and R-square analysis were used for selection of adequacy models (Table 3 to 5), as outlined by Lee and others (2000), Weng and others (2001), and Chen and others (2003). Table 3 compares the validities of linear, quadratic, and cubic models for the 3 responses according to their F values. A model with P values ($P > F$) below 0.05 is regarded as significant. The highest order polynomial that is significant is selected. The lack-of-fit test (Table 4) compares the residual and pure errors at replicated design points. If there is a significant lack of fit, as indicated by a low probability value ($P > F$), the response predictor should be discarded. The model with no significant lack of fit is selected. ANOVA demonstrates that Eq. 6 and 7 (quadratic model for the growth rate of *Lac-*

tobacillus spp. and 2 cubic models for the hardness and *Bifidobacteria* spp., respectively) appear to be the most accurate, with no significant lack of fit (Table 3 to 5).

$$f_1 = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad (6)$$

$$f_r = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j + \sum_{i=1}^{n} \beta_{iii} X_i^3 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{iij} X_i^2 X_j + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ijj} X_i X_j^2 + \sum_{i=1}^{n-2} \sum_{j=i+1}^{n-1} \sum_{k=j+1}^{n} \beta_{ijk} X_i X_j X_k$$

$$r = 2, 3 \quad (7)$$

where n is the number of independent variables ($n = 4$); f_1 and f_2 are the growth rates of *Lactobacillus* spp. and *Bifidobacteria* spp., respectively, and f_3 is the hardness of the product; βs are regression coefficients, and Xs are the uncoded independent variables. The regression coefficients for the statistically significant models are given in Table 3 to 5. The 3-level BBD design is incapable of forming the pure cubic terms (that is, $\beta_{iii}X_i^3$ in Eq. 7), and the coefficients presented in Table 6 confirming this fact. The 3 responses are then combined into 1 single function (CF) whose maximum can then be sought by optimization techniques.

Optimizing ingredient combinations

Because the composite function is a product of 1 quadratic and 2 cubic functions, it appears very likely that multiple local maxima exist. Therefore, a global optimization program consisting of a multi-start SQP was coded, with the probability criterion (Eq. 5) for a CF to achieve a global optimum. The program generates a series of uniformly distributed random points as initial search points, and then SQP is applied to find the optimum based on each subsequent initial point. If the probability exceeds a preset value (99.99% in this study) according to Eq. 5, the global optimum is considered found. Otherwise, the next random initial point is generated and the SQP re-executed. Figure 1 depicts the evolution of all optimal values graphically. In Figure 1, 5 different local optimal CF values (0.98 to 1.47) were identified from 56 randomly generated initial points. Of these local optima, the global optimal CF value was 1.47 (99.99% certainty). The optimal CF values correspond to 0.20 log CFU/h of the growth rate for *Lactobacillus* spp.; 0.23 log CFU/h of the growth rate for *Bifidobacteria* spp.; and a product hardness of 56.23 g. The highest optimal CF value (1.47) was attained for 12 of 56 sets, with the optimal points, X1 = 1, X2 = 0, X3 = 3, and X4 = 18. The optimal conditions for manufacturing probiotic dairy tofu were 18% skim milk blended with 1% GDL, 0% peptides, and 3% IMO.

It is well known that increasing the total solid content of milk increases the firmness and viscosity of the yogurt. Pereira and others (2003) have studied the textural characteristics of acid milk gels. They confirmed that gel firms considerably as total solids increase, with a decrease in pore size and an increase in the degree of interconnectivity between the clusters of protein. In this study, the highest skim-milk concentration suggested by the optimization method concurs with the above research.

The probiotics growth rate could be affected by the pH of the acidified milk before inoculation and the addition of prebiotics to the milk. Shah and Jelen (1990) concluded that the main factors for loss of viability of probiotic organisms were the decrease in the pH of the medium and accumulation of organic acid. Because the probiotics were inoculated immediately after the GDL was blended at 37 °C and the GDL hydrolyzes slowly at low temperature, the effects of pH of the acidified milk before inoculation were excluded.

Table 2—Box-Behnkin design matrix with 3 responses

Independent variables				Responses		
GDL%	Peptide%	IMO%	Milk%	GR[a](L)[a]	GR(B)[a]	Hardness[a]
0	0	0	0	0.21	0.12	29.25
0	–1	1	0	0.20	0.19	39.56
0	0	1	1	0.24	0.17	22.95
1	0	1	0	0.25	0.14	37.34
0	0	0	0	0.22	0.16	19.45
–1	1	0	0	0.22	0.14	9.95
1	0	0	–1	0.21	0.13	38.13
0	1	1	0	0.25	0.19	19.66
0	–1	0	–1	0.18	0.18	36.36
1	1	0	0	0.22	0.12	41.39
0	0	0	0	0.23	0.18	21.68
0	0	0	0	0.23	0.16	25.13
–1	0	1	0	0.24	0.16	14.26
1	0	0	1	0.21	0.15	31.66
0	–1	0	1	0.18	0.14	54.48
–1	0	0	–1	0.24	0.18	22.32
0	1	0	1	0.22	0.17	20.12
–1	0	0	1	0.24	0.16	11.33
–1	0	–1	0	0.23	0.18	11.10
0	0	0	0	0.23	0.15	26.09
0	0	–1	–1	0.22	0.20	16.8
0	1	0	–1	0.22	0.20	25.21
0	1	–1	0	0.24	0.22	25.29
–1	–1	0	0	0.20	0.19	14.84
1	0	–1	0	0.21	0.17	46.32
0	0	1	–1	0.23	0.16	48.51
0	–1	–1	0	0.22	0.15	27.04
1	–1	0	0	0.19	0.22	55.11
0	0	–1	1	0.21	0.21	11.82

[a]GR = growth rate; L = *L. acidophilus + L. casei*; B = *B. longum + B. bifidum*; Hardness = unit in grams.

The pH values of skim milk with GDL were still around 6.6 to 6.7 before inoculation. On the other hand, in our study, prebiotics did affect the growth rate of probiotics. Fooks and others (1999) have revealed that IMO are very efficient prebiotic agents in that they are able to stimulate lactic microflora as well as facilitate the elevated production of butyrate, thought to be a desirable metabolic in the gut. The current study confirms that IMO improves probiotic growth rate. A number of earlier studies have investigated the ef-

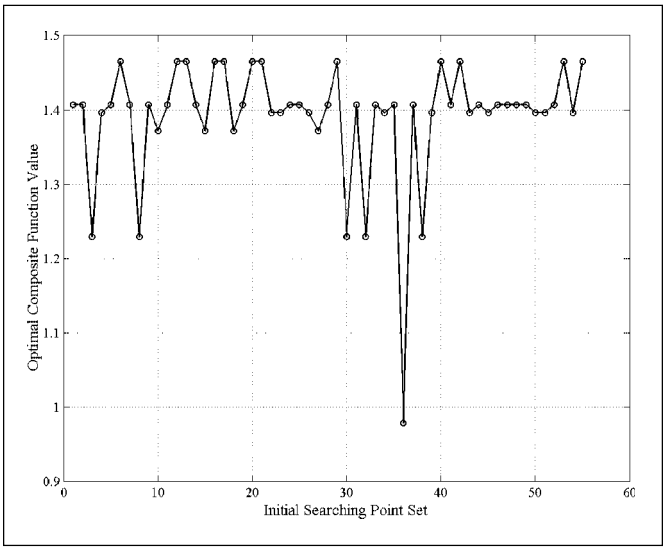

Figure 1—Optimum CF values for randomly generated initial searching points when using SQP

Table 3—Model analysis[a] of probiotic growth rate model and hardness model of probiotics dairy tofu[b]

	L[c] growth rate		B[c] growth rate		Hardness	
Source	Sum of squares	*P* > F	Sum of squares	*P* > F	Sum of squares	*P* > F
Mean	1.40		0.82		22240.55	
Linear	4.003×10^{-3}	0.0038**	4.977×10^{-3}	0.0491*	3176.33	< 0.0001**
Quadratic	3.134×10^{-3}	0.0342*	6.419×10^{-3}	0.0286*	610.08	0.0464*
Cubic	9.400×10^{-4}	0.4140	1.257×10^{-3}	0.0115*	838.29	0.0306*
Residual	5.758×10^{-4}		4.544×10^{-4}		122.27	
Total	1.41		0.029		26987.52	

[a]Model analysis: select the highest order polynomial where the additional terms are significant.
[b]*Significant at 5% level; **significant at 1% level.
[c]L = *L. acidophilus + L. casei.*; B = *B. longum + B. bifidum.*

Table 4—Lack-of-fit test[a] of probiotic growth rate model and hardness model of probiotics dairy tofu[b]

	L[c] growth rate		B[c] growth rate		Hardness	
Source	Sum of squares	*P* > F	Sum of squares	*P* > F	Sum of squares	*P* > F
Linear	4.497×10^{-3}	0.0486*	0.017	0.3752	1511.74	0.0618
Quadratic	1.363×10^{-3}	0.1154	0.011	0.2857	901.66	0.0578
Cubic	4.233×10^{-4}	0.0701	4.776×10^{-4}	0.6804	63.37	0.2321
Pure error	1.525×10^{-4}		2.249×10^{-3}		58.90	

[a]Lack-of-fit test: want the selected model to have insignificant lack-of-fit.
[b]*Significant at 5% level.
[c]L = *L. acidophilus + L. casei.*; B = *B. longum + B. bifidum.*

Table 5—R-square analysis of probiotic growth rate model and hardness model of probiotics dairy tofu

	L[a] growth rate	B[a] growth rate	Hardness
Source	R-square	R-square	R-square
Linear	0.4626	0.0943	0.6691
Quadratic	0.8248	0.3971	0.7976
Cubic	0.9335	0.8714	0.9742

[a]L = *L. acidophilus + L. casei*; B = *B. longum + B. bifidum.*

Table 6—The coefficients of probiotic growth rate model and hardness model of probiotic dairy tofu

Coefficient	GR (L)[a]	GR (B)[a]	Hardness
β_0	0.12	0.59	–71.32
β_1	–0.028	–0.61	169.00
β_2	0.026	–0.33	224.30
β_3	–0.035	0.042	19.42
β_4	0.017	–0.033	1.46
β_{11}	0.011	0.42	–79.31
β_{22}	–0.022	0.13	–100.26
β_{33}	5.564×10^{-3}	-3.152×10^{-3}	–7.43
β_{44}	-6.052×10^{-4}	5.912×10^{-4}	0.21
β_{12}	7.010×10^{-4}	0.47	–171.35
β_{13}	0.012	–0.074	59.22
β_{14}	-1.658×10^{-3}	0.020	–10.47
β_{23}	6.446×10^{-3}	–0.074	1.34
β_{24}	1.095×10^{-3}	0.020	–12.26
β_{34}	7.064×10^{-4}	-5.358×10^{-5}	–1.14
β_{111}	0	0	0
β_{222}	0	0	0
β_{333}	0	0	0
β_{444}	0	0	0
β_{112}	0	–0.50	73.22
β_{113}	0	0.027	–66.23
β_{114}	0	-8.514×10^{-3}	8.88
β_{122}	0	0.088	45.17
β_{133}	0	9.675×10^{-3}	7.04
β_{144}	0	0	0
β_{223}	0	0.025	–10.65
β_{224}	0	–0.013	6.45
β_{233}	0	6.878×10^{-3}	3.53
β_{244}	0	0	0
β_{334}	0	0	0
β_{344}	0	0	0
β_{123}	0	0	0
β_{124}	0	0	0
β_{123}	0	0	0
β_{134}	0	0	0
β_{234}	0	0	0

[a]GR = growth rate; L = *L. acidophilus + L. casei*; B = *B. longum + B. bifidum.*

fects of peptides upon human gut bacteria (Mitsuoka and others 1987; Dave and Shah 1998; Lourens-Hattingh and Viljoen 2001). Nitrogen sources, in the form of various peptides and amino acids, probably act by improving the viability of the bifidobacteria present in the gut (Lourens-Hattingh and Viljoen 2001). This study did not confirm the prebiotic effects of peptide, however.

To further depict the global optimization results, 3-D response surface plots were generated by fixing 2 of the 4 factors. Figure 2a shows 4 local maxima, including the global analog, in a CF response function produced by setting X2 = 0 and X4 = 18, while varying X1 and X3 within their boundaries. Figure 2b shows the other 3 local maxima determined during the optimization process by fixing X1 = 1 and X3 = 3, while changing X2 and X4. The optimization results clearly show that whether the global optimum can be found depends on the initial search points for our response surface models.

Experimental verification

The optimal production conditions were derived using SQP and verified by independent additional experiments. The optimal manufacturing conditions were GDL (1%), IMO (3%), and milk (18%), in combination with minimum peptide (0%). The 3 responses, the growth rates for *Lactobacillus* spp. and *Bifidobacteria* spp. and the hardness of the dairy tofu, and the composite function value derived from the verification experiments were all very close to the SQP-predicted values, with no apparent significant differences ($P > 0.05$) being demonstrated comparing the 2 sets (Table 7).

Microstructure, sensory evaluation, and storage test

The probiotic GDL tofu was obtained by coagulation of milk with addition of GDL and bacterial culture, while the yogurt was acidified by using bacterial culture. Because GDL tofu (coagulation of soy milk by GDL), plain yogurt (coagulation of milk by fermented cultures),

GDL-acidified milk gel (coagulation of milk by GDL), and our new probiotic dairy tofu (coagulation of milk by fermented cultures and GDL) have common characteristics (at least partially), the microstructures and sensory characteristics of them were compared in this study. GDL tofu, which was made at 80 °C, has a final pH value of 5.7 and protein content of 5.86%. The final pH and protein content of probiotic tofu, GDL-acidified milk gel and yogurt are similar (pH 4.4 to 4.5, the crude protein 5.72% to 5.81%). The latter gels were made at 37 °C.

The SEM image of GDL tofu (Figure 3a) shows the network structures constructed with small protein granules. It had well-developed protein aggregations and connections between protein granules. Soybean seeds have a protein content of 35% to 40% on a dry weight. Tofu is actually a precipitated soy protein, and it starts coagulating at pH 6.0 (Kim and Han 2002). The GDL-acidified milk gel (Figure 3b) shows a coarse particulate network of casein particles linked together in clusters, chains, and strands. GDL causes milk proteins to aggregate due to pH reduction. Comparing the microstructures of the probiotic dairy tofu (Figure 3d) and yogurt (Figure 3c), relatively open and large pores are observed in the microstructure of the yogurt gels. The probiotic dairy tofu is characterized by a finer-meshed protein network, more cluster interconnectivity, and smaller pore size. The microstructure of yogurt has been described as three-dimensional network of chains and clusters of casein micelles retaining their globular shapes. Lucey and others (1998) have compared GDL-induced gels and yogurt and concluded that the former appears to have numerous cross-links and a high degree of interconnectivity. Bacterial gels are much more tortuous with less apparent cross-linking than GDL-induced analogs.

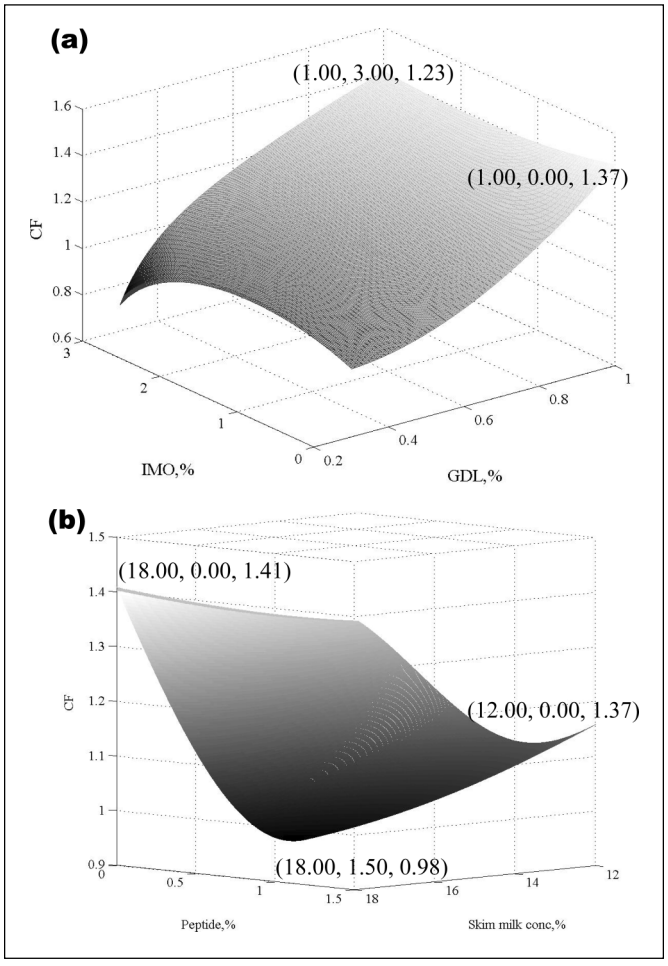

Figure 2—A response surface plot under the conditions of constant (a) peptide (0%) and skim milk (18%) and (b) GDL (1.0%) and IMO (3%)

Table 7—The validation of the optimum producing models of probiotic dairy tofu recommended by the SQP techniques

	L[a] growth rate (logCFU/mL/h)		B[a] growth rate (logCFU/mL/h)		Hardness (g)	
	Pred[a]	Exp[a]	Pred[a]	Exp[a]	Pred[a]	Exp[a]
SQP	0.20	0.20	0.23	0.24	56.23	55.52

[a]L = *L. acidophilus + L. casei;* B = *B. longum + B. bifidum;* Pred = predicted value; Exp = experimental value. Note: The predicted and experimental values for the same responses have no significant difference ($P > 0.05$).

Table 8—Sensory evaluations of GDL tofu, plain yogurt, and probiotic dairy tofu

Item[a]	GDL tofu[b]	Plain yogurt[b]	Dairy tofu[b]
Appearance	8.00a	6.85b	8.00a
Body/Texture	8.00a	5.79b	7.98a
Aroma/taste	8.70a	7.24b	5.13c

[a]Scored in a one-to-nine hedonic scale, where 1 is for "extremely poor" and 9 for "excellent."
[b]Values in the same row without a letter are significantly different ($P < 0.05$).

The sensory evaluation results revealed no significant difference between the probiotic dairy tofu and the GDL tofu in texture and appearance, which were rated as good to excellent by the judges ($P > 0.05$; Table 8). However, the probiotic dairy tofu was sour and it had the lowest score for aroma/taste ($P < 0.05$).

The viability of the probiotics, in terms of probiotic count, were examined every wk during 4 wk of storage at 4 °C (Figure 4). The *Lactobacillus* spp. count increased with storage time during the 1st 2 weeks, with final viable counts maintained at 9.0 log CFU/mL. Conversely, the number of *Bifidobacterium* spp. decreased with storage time, and final viable count was 8.0 log CFU/mL. In general, for probiotic dairy tofu, the viable numbers were well above the recommended limit of 1 million g^{-1} throughout the storage period. This could be due to the addition of the prebiotics and/or the type of probiotic strain (Lankaputhra and others 1996).

Conclusions

In this study, we have demonstrated the robustness of a two-stage process for derivation of a surface model using response surface methodology and SQP optimization. The optimal conditions, 1% GDL, 0% peptides, 3% IMO, and a milk concentration of 18%, were subsequently confirmed by verification experiments. Analysis of the sensory-evaluation results reveals no significant differences between the probiotic dairy tofu and the GDL product in terms of texture and appearance ($P > 0.05$). The viable numbers remained well above the recommended limit of 10^6 CFU/ g throughout the storage period for the probiotic dairy tofu.

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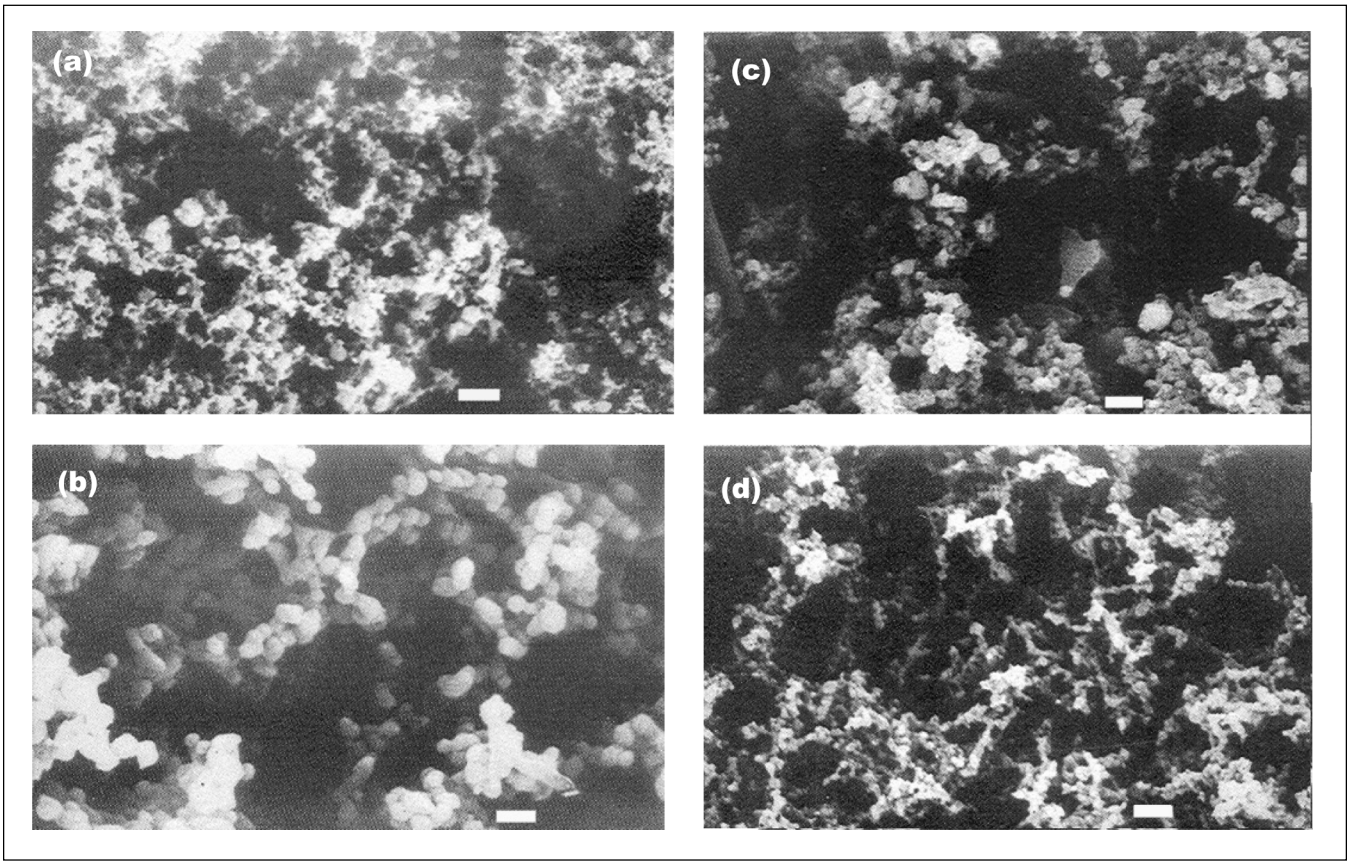

Figure 3—The microstructures of (a) GDL tofu, (b) GDL acidified milk gel, (c) plain yogurt, and (d) probiotic dairy tofu. Bar = 1 μm.

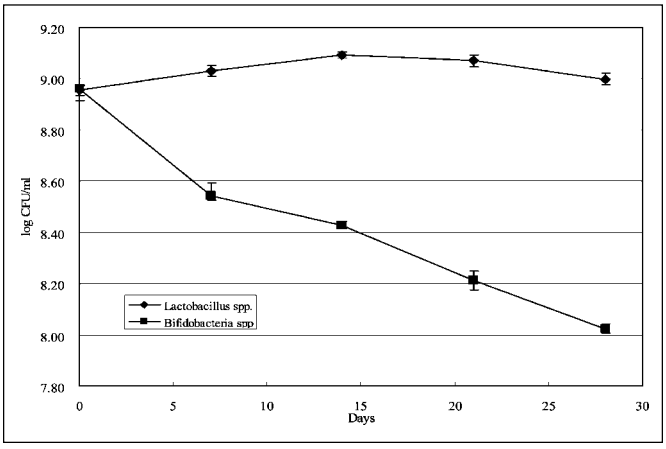

Figure 4—The probiotics of an optimal probiotic dairy tofu during storage at 4 °C

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