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Journal of Food Engineering 68 (2005) 471–480
JOURNAL OF FOOD ENGINEERING
www.elsevier.com/locate/jfoodeng

Optimization on response surface models for the optimal manufacturing conditions of dairy tofu

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Received 2 January 2004; accepted 29 June 2004

Abstract

The purpose of this research was to create response surface models through regression on experimental data and to apply the Sequential Quadratic Programming (SQP) and Genetic Algorithms (GAs) on the models to obtain optimal processing conditions for dairy tofu. The two-stage effort of obtaining a surface model using response surface methodology (RSM), and optimizing this model using GAs or SQP techniques was demonstrated to be an effective approach. Both SQP and GAs techniques were able to determine the optimal conditions for manufacturing the probiotic dairy tofu. The conditions were 1% of glucono-delta-lactone (GDL), 0% of peptides level, 3% of isomaltooligosaccharides (IMO) and 18% of milk concentrations, and they were confirmed by verification experiments. Among the SQP and two GAs employed, the SQP, modified with the multi-start capability, is the most efficient one.

1. Introduction

In recent years, there has been a worldwide increase in consumption of fermented milk, especially for those products with probiotics. Developing the new dairy products with probiotics could provide the varieties of selections for customers and might robust the market in dairy industry (Liu, Chen, & Lin, 2002). The idea of probiotic dairy tofu was from glucono-delta-lactone (GDL) tofu and yogurt. This new product, containing probiotics and prebiotics, provides the texture of tofu, flavor of yogurt, as well as health benefits. In order to manufacture a good quality dairy tofu and understand the effect of different ingredients on 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 contains three stages (Myers & Montgomery, 1995): (1) design of experiments, (2) response surface modeling through regression, and (3) optimization. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions (Lee, Ye, Landen, & Eitenmiller, 2000; Porretta, Birzi, & Vicini, 1995). The experimental data were utilized to build mathematical models using regression methods. Once an appropriate approximating model is obtained, this model can then be analyzed using various optimization techniques to determine the optimum conditions for the process. RSM was successfully used for applications in developing new edible gels (Chen & Lin, 2002) and finding the optimum producing

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doi:10.1016/j.jfoodeng.2004.06.028

conditions of the dairy product Kou Woan Lao, which is produced by mixing milk and culture filtrates extracted from a fermented rice product and used to form milk curds and to enhance flavor (Weng, Liu, & Lin, 2001).

Optimization theory consists of a body of numerical methods for finding and identifying the best candidate from a collection of alternatives without having to explicitly evaluate all possible alternatives (Reklaintis, Ravindran, & Ragsdell, 1983). In the context of RSM, empirical (mathematical) models are built using regression techniques on the results of a selected set of experiments. A well fitted model represents, approximately, all possible experiments with their experimental factors within the preset bounds. Through the use of optimization techniques, the optimum of the model corresponding to the experiment with conditions that will presumably produce the best result can thus be found. The final step is to perform experimental verification based on the optimal, experimental conditions. Among the optimization techniques, the steepest ascent (or descent) is commonly used (see, for example, State-Ease, Inc., 2000), but the method is relatively inefficient and is a local optimization technique capable of finding only local optima. Genetic Algorithms (GAs), although even less efficient than the steepest ascent, are considered as global schemes. The Sequential Quadratic Programming (SQP) technique is very powerful and efficient, and with some modifications it can also perform global optimizations (Chen, 2003). Both SQP and GAs were studied in this research.

Genetic Algorithms are search procedures that imitate the natural evolution process and can be used for the computation of the global maximum or minimum of a function (Mitchell, 1996). Genetic Algorithms differ from other search techniques in that they search among a population of points and use probabilistic rather than deterministic transition rules. As a result, Genetic Algorithms search more globally (Wang, 1997). D'souza and Simpson (2003) utilized the so-called the non-dominated sorting Genetic Algorithm for product family design and optimization. Chen, Chen, and Lin (2003) optimized the viability of probiotics in a new fermented milk drink and concluded that the two-stage effort, obtaining a surface model and optimizing this model using the GAs, resulted in a useful method of finding an optimal set of process parameters.

A quadratic programming problem is an optimization problem involving a quadratic objective function and linear constraints. The Sequential Quadratic Programming method represents state-of-the-art in nonlinear programming methods (The Math Works Inc., 2000) and can be used to solve a series of quadratic programming problems approximating the original nonlinearprogramming problem. The SQP is a powerful tool but involves a complicated procedure. The theory behind the SQP can be found in most optimization textbooks, e.g. Arora (1989) and Haftka and Gürdal (1992), and will not be elaborated here.

The purpose of this research was to create response surface models through regression on experimental data and to apply the SQP and GAs on the models to obtain optimal processing conditions for dairy tofu.

2. Experimental process and response surface modeling

This chapter describes the pre-optimization stages, including design of experiments, experimental process and response surface modeling. Optimizations will be presented in the next chapter.

2.1. Experimental process

The dairy product under investigation is a new type of dairy tofu made from milk. The experimental process is described in the following subsections.

2.1.1. Preparation of probiotic dairy tofu

The samples were prepared using 12–18% (w/w) skim milk powder (Anchor Foods, New Zealand, protein 37.60%, lactose 49.80%). Reconstituted skim milk in deionized water was mixed with 0.3–1% of gluconodelta-lactone (GDL) and the prebiotics (peptides from casein, pancreatic digested, 0.0–1.0%, Cheng-Fung Co., Taiwan; isomaltooligosaccharides, IMO, 0.0–3.0%, Cheng-Fung Co., Taiwan). Then, the mixed samples (each of 200 mL) were inoculated with 1% each of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacteria bifidum* and *Bifidobacteria longum* in 250 mL beakers covered with aluminum foil, and fermented for 12 h at 37 °C. The fermentation time and temperature were suggested by the preliminary tests, which revealed that dairy tofu with 12-h fermentation at 37 °C yielded better hardness and had higher viability of probiotics. The headspace volume of the fermentation was around 20% of the total volume.

2.1.2. Cultures and medium performance

Pure lyophilized cultures of *B. longum* (CCRC 14605), *L. casei subsp. rhamnosus* (CCRC 12321), *B. bifidum* (CCRC 11844) *L. acidophilus* (CCRC 14079) were purchased from the Culture Collection and Research Center, Hsinchu, Taiwan, ROC 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, Undeland, & Cox, 1992).

2.1.3. Determination of probiotic growth rate

For the determination of the viabilities of the probiotics, the populations of *Lactobacillus* spp. and

bifidobacteria spp. were measured as growth rates. 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 were incubated anaerobically (72 h at 37 °C, GasPak System-Oxoid, Basingstoke, Hampshire, England). 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:

$$\mathrm{GR} = \frac{[\log(\mathrm{CFU}_1) - \log(\mathrm{CFU}_2)]}{t_2 - t_1} \quad (1)$$

where CFU_1 and CFU_2 are the CFU at time t_1 (fermentation for 0 h) and t_2 (fermentation for 12 h).

2.1.4. Determination of hardness

The hardness of samples was determined by testing 5 replicate samples on a TA-XT2i/5 Texture Analyser (Stable Micro Systems, USA) fitted with a 5 kg load cell. The gels were formed in glass containers (50 mm diameter, 65 mm height) with 80 mL of mixed samples and tested using a cylinder probe with a flat-ended head of 20 mm in diameter at a fixed rate of 10 mm/s. 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.

2.2. Response surface modeling

Before any experiment taking place, design of experiments were first performed. The Box and Behnken design (BBD) (Box & 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 three evenly spaced levels (Myers & Montgomery, 1995). A four-variable BBD with five replicates at the center point was selected to build response surface models. The coded and uncoded variables and their respective levels are shown in Table 1.

To carry out response surface modeling, the regression method was performed on experimental results to build mathematical models. The models were then formulated as an objective function in an optimization problem that was consequently optimized using optimization techniques to obtain the maximum viability of the probiotics and the maximum hardness of the product. The RSM procedure of the Design-Expert® software package (State-Ease, Inc., 2000) was used to fit the experimental data to polynomial equations of order one through three to obtain coefficients. The following linear relationship achieved this.

Table 1
Process variables and their levels in four variables-three 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

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

where Y_1, Y_2, Y_3 were the growth rates of *Lactobacillus* spp. and *Bifidobacteria* spp. and the hardness of the dairy tofu, respectively. f_1, f_2, f_3 represented the modeled response surfaces. X_1, X_2, X_3, X_4, defined as natural variables, were the concentrations of GDL, peptides, IMO and milk,respectively. ϵ_1, ϵ_2, ϵ_3 were the errors in each model. With RSM, it is convenient to transform the natural variables to coded variables ξ_1, ξ_2, ξ_3, ξ_4, where the coded variables are defined as dimensionless, with mean zero and the same spread or standard deviation:

$$Y_i = f_i(\xi_1, \xi_2, \xi_3, \xi_4) + \epsilon_i \quad i = 1, 2, 3 \quad (3)$$

3. Optimization on the response surface models and model verifications

3.1. Development of the objective function

All measured variables are subjected to random errors and should be considered in the formulation of the objective function. In order to search a solution maximizing multiple responses, a composite function (CF) was defined as the following:

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

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

3.2. Optimization by the sequential quadratic programming

An SQP procedure implemented in the MATLAB (The Math Works Inc., 2000) environment was employed to optimize the growth rate of probiotics and hardness of probiotic dairy tofu that were formulated, via RSM, as polynomial functions of four independent variables bounded by preset upper and lower limits. The basic scheme of an SQP technique can be expressed in the following steps (Chen, 2003; Reklaintis et al., 1983):

Step 1: Set up and solve a quadratic programming (QP) subproblem, giving a search direction.
Step 2: Test for convergence, stop if it is satisfied.
Step 3: Step forward to a new point along the search direction.
Step 4: Update the Hessian matrix in QP and go to step 1.

In order to search for the global optimum, the concept of multi-start global optimization procedure (Snyman & Fatti, 1987) was combined with the SQP method. 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 be equal to F^* satisfies the following relationship (Chen, 2003):

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

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

3.3. Optimization by Genetic Algorithms

In the present paper, the Simple Genetic Algorithm and the Micro Genetic Algorithm were also employed to optimize the processing conditions. Both GAs were programmed in MATLAB (The Math Works Inc., 2000) codes. The numbers of bits in the binary strings, population size, crossover rate, mutation rate and maximum number of generations in GAs were all obtained by fine tuning the algorithms through multiple trial runs, which is a common practice for using GAs to solve an optimization problem, e.g. D'souza and Simpson (2003) and Vallapuzha et al. (2002).

3.3.1. Simple Genetic Algorithm

The Simple Genetic Algorithm (SGA)(Chen et al., 2003; Mitchell, 1996) searches for optimal values by simulating the biological evolutionary process, based on crossover and mutational genetics. In order to use the GAs, a chromosome was formed by all four independent variables, i.e. four ingredients: concentrations of GDL, peptides, IMO and milk, which were all coded as 20-bit binary strings making an 80-bit chromosome. Table 2 shows the parameters for the SGA. The initial population, consisting of 50 chromosomes (population size), was generated at random. The crossover and mutation operators were applied to those chromosomes. The crossover rate and mutation rate were 0.5 and 0.02 individually. The selection technique was based on the roulette wheel selection and the elitist strategy (Chen et al., 2003; Mitchell, 1996). The roulette wheel technique is the most simple selection method while the elitist strategy makes sure the one chromosome with the highest CF value survives to the next generation. The maximum number of generations was set to 100 for SGA.

This iterative process continues until a pre-specified maximum number (100) of generations are reached, or until there is no appreciable improvement in the CF value. With each new generation, the population gets closer to an optimal value. Once the search is complete, the best value from the final generation is taken as the optimal solution.

3.3.2. Micro Genetic Algorithm

The essence of the Micro Genetic Algorithm (MGA) (Chen et al., 2003) is the lack of mutations and the presence of restarts. Due to these features, the algorithm converges rapidly to a local or global maximum (Nikitas, Pappa-Louisi, Papageorgiou, & Zitrou, 2001). The lack of mutations also results rapidly in a decrease of the variance of the cost values of the population. When the variance value falls below a certain limit, a restarting process begins, in which the chromosome with the highest CF value is retained and the rest $N-1$ chromosomes (N is the total number of chromosomes in one generation) are replaced by randomly generated new ones.

Table 2 also shows the parameters for the adopted MGA. The initial population consisting of 10 chromosomes (population size) was generated at random. The crossover rate was 0.5. The chromosomes with higher CF values were selected and retained for the next

Table 2
Parameters of the Simple Genetic Algorithm (SGA) and the Micro Genetic Algorithm (MGA)

Parameter	SGA	MGA
Population size	50	10
Number of bits[a]	20×4	20×4
Mutation rate	0.02	0
Crossover rate	0.5	0.5
Maximum generation	100	500

[a] No. of bits = No. of bits per variable × No. of variables.

generation. The maximum number of generations was set to 500 for the MGA.

3.4. Model verification

After optimal processing conditions were found by the SQP and GAs, experiments based on the conditions were performed and repeated three times. The results were then analyzed using ANOVA from the SAS software package (SAS Institute Inc., 1990), with Duncan's multiple range test for significance to detect differences between predicted values and observed values.

4. Results and discussion

4.1. Response surface modeling

The present work has developed prediction models for the growth rates of probiotics and the hardness of the probiotic dairy tofu by using RSM. Four treatments (concentrations of GDL, peptides, IMO and milk) were mixed with milk in an attempt to improve the growth rate of *Lactobacillus* spp. and *Bifidobacteria* spp. as well as the hardness of the product.

The experimental results for the probiotic growth rates and hardness of the probiotic dairy tofu based on a Box–Behnkin design of experiments, and analysis of variance (ANOVA) of their means are presented in Tables 3 and 4. According to the results, approximate functions are constructed using a curve fitting procedure. The model-fitting step was carried out using the Design-Expert® software package, which employs the least squares procedure to compute the model coefficients. The responses modeled as linear, quadratic and cubic functions of the four independent variables were tested for adequacy and for model fitness using ANOVA. The selections of adequate models (Table 4) were determined using model analysis, lack-of fit test and *R*-square analysis as outline by Lee et al. (2000) and Weng et al. (2001). Table 4(a) examines the probability (Prob > F) to see if it falls below 0.05. The highest order polynomial that is significant is selected. The "Lack of Fit Test" (Table 4b) compares the residual error to the

Table 3
Box–Behnkin design matrix with three responses

Independent variables				Responses		
GDL%	Peptide%	IMO%	Milk%	GR[a] (L)[b]	GR(B)[c]	Hardness[d]
0	0	0	0	0.153 ± 0.005	0.124 ± 0.004	36.18 ± 3.24
0	−1	1	0	0.145 ± 0.004	0.163 ± 0.005	64.42 ± 5.87
0	0	1	1	0.174 ± 0.003	0.125 ± 0.003	37.60 ± 2.03
1	0	1	0	0.173 ± 0.007	0.123 ± 0.007	48.86 ± 4.33
0	0	0	0	0.165 ± 0.006	0.124 ± 0.006	41.18 ± 4.32
−1	1	0	0	0.192 ± 0.006	0.123 ± 0.003	23.12 ± 5.73
1	0	0	−1	0.131 ± 0.009	0.116 ± 0.004	44.90 ± 3.21
0	1	1	0	0.162 ± 0.006	0.133 ± 0.005	32.45 ± 2.45
0	−1	0	−1	0.134 ± 0.009	0.124 ± 0.003	54.17 ± 5.12
1	1	0	0	0.152 ± 0.003	0.135 ± 0.007	48.86 ± 1.01
0	0	0	0	0.175 ± 0.006	0.123 ± 0.009	32.32 ± 3.98
0	0	0	0	0.166 ± 0.004	0.107 ± 0.003	39.99 ± 2.39
−1	0	1	0	0.174 ± 0.003	0.124 ± 0.002	28.89 ± 3.25
1	0	0	1	0.153 ± 0.005	0.116 ± 0.003	54.30 ± 3.45
0	−1	0	1	0.133 ± 0.007	0.134 ± 0.002	82.70 ± 2.11
−1	0	0	−1	0.183 ± 0.005	0.115 ± 0.003	29.24 ± 3.53
0	1	0	1	0.156 ± 0.007	0.117 ± 0.001	35.86 ± 4.21
−1	0	0	1	0.195 ± 0.007	0.123 ± 0.003	37.15 ± 5.23
−1	0	−1	0	0.183 ± 0.006	0.138 ± 0.003	36.64 ± 3.27
0	0	0	0	0.163 ± 0.008	0.104 ± 0.002	39.59 ± 2.31
0	0	−1	−1	0.175 ± 0.005	0.135 ± 0.001	42.59 ± 4.54
0	1	0	−1	0.153 ± 0.005	0.103 ± 0.002	33.81 ± 2.32
0	1	−1	0	0.187 ± 0.006	0.165 ± 0.001	43.36 ± 3.17
−1	−1	0	0	0.156 ± 0.007	0.134 ± 0.002	49.30 ± 3.02
1	0	−1	0	0.178 ± 0.003	0.124 ± 0.002	58.15 ± 4.35
0	0	1	−1	0.165 ± 0.004	0.123 ± 0.003	56.35 ± 1.43
0	−1	−1	0	0.143 ± 0.007	0.146 ± 0.002	61.74 ± 2.23
1	−1	0	0	0.145 ± 0.005	0.154 ± 0.004	77.90 ± 3.48
0	0	−1	1	0.184 ± 0.006	0.103 ± 0.005	39.25 ± 1.32

[a] GR: growth rate.
[b] L: *L. acidophilus* + *L. casei*.
[c] B: *B. longum* + *B. bifidum*.
[d] Hardness: Unit in g.

Table 4
(a) Model analysis, (b) lack of fit and (c) *R*-square analysis of probiotic growth rate model after 12-h fermentation

Source	AC[a] growth rate		B[b] growth rate		Hardness	
	Sum of squares	$P > F$	Sum of squares	$P > F$	Sum of squares	$P > F$
(a) Model analysis[c]						
Mean	0.74	0.0030**	0.43	0.0021**	59256.57	<0.0001**
Linear	4.930×10^{-3}	0.0002**	5.967×10^{-4}	0.6469	3936.02	<0.0001**
Quadratic	2.506×10^{-3}	0.0212*	3.996×10^{-3}	0.0211*	989.09	0.0924
Cubic	7.527×10^{-4}	0.2476	8.156×10^{-4}	0.6943	583.00	0.0135*
Residual	3.159×10^{-4}		8.862×10^{-4}		60.67	
Total	0.75		0.44		64825.35	
(b) Lack of fit[d]						
Linear	3.447×10^{-3}	0.0561	5.280×10^{-3}	0.1906	1580.61	0.0463*
Quadratic	9.416×10^{-4}	0.1531	1.284×10^{-3}	0.4553	591.52	0.0790
Cubic	1.889×10^{-4}	0.1615	4.684×10^{-4}	0.2222	8.52	0.7387
Pure error	1.270×10^{-4}		4.178×10^{-4}		52.14	
(c) R-square analysis[e]						
	R-square	Press	*R*-square	Press	*R*-squared	Press
Linear	0.5797	5.538×10^{-3}	0.0948	8.562×10^{-3}	0.7068	2399.16
Quadratic	0.8743	5.622×10^{-3}	0.7296	8.049×10^{-3}	0.8844	3488.66
Cubic	0.9629	0.027	0.8592	0.068	0.9891	1309.02

*Significant at 5% level.
**Significant at 1% level.
[a] AC: *L. acidophilus* + *L. casei.*
[b] B: *B. longum* + *B. bifidum.*
[c] Model analysis: select the highest order polynomial where the additional terms are significant.
[d] Lack of fit test: want the selected model to have insignificant lack-of-fit.
[e] *R*-square analysis: focus on the model minimizing the "Press".

pure error from replicated design points. If there is a significant lack of fit, as indicated by a low probability value (Prob > F), the response predictor should be discarded. The model with insignificant lack-of-fit is selected. ANOVA showed that the quadratic models of Eq. (6) for the growth rates of *Lactobacillus* spp. and *Bifidobacteria* spp. as well as the cubic model of Eq. (7) for the hardness of the product appeared to be the most accurate, with no significant lack of fit (Table 4).

$$f_k = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad k = 1, 2 \tag{6}$$

$$f_3 = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j + \sum_{i=1}^{n} \beta_{iii} X_i^3 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{iij} X_i^2 X_j + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ijj} X_i X_j^2 + \sum_{i=1}^{n-2} \sum_{j=i+1}^{n-1} \sum_{k=j+1}^{n} \beta_{ijk} X_i X_j X_k \tag{7}$$

where f_1 and f_2 were the growth rates of *Lactobacillus* spp. and *Bifidobacteria* spp., respectively, and f_3 was the hardness of the product. β_0, β_i, β_{ii}, β_{ij}, β_{iii}, β_{iij}, β_{ijj}, β_{ijk} were constant coefficients and X_i, X_j, X_k were the uncoded independent variables. The regression coefficients for the statistically significant models are given in Table 5. The three-level design of BBD is incapable of forming the pure cubic terms, i.e. $\beta_{iii} X_i^3$ in Eq. (7), and the coefficients in Table 5 do concur with this fact. The three responses were then combined into one single function (Composite Function) whose maximum can then be sought by optimization techniques.

4.2. Search for optimal combinations of ingredients

4.2.1. Sequential quadratic programming

Since the composite function was a product of two quadratic and one cubic functions, it was very likely that there existed more than one local maximum. Therefore, a global optimization program consisting of a multi-start SQP with the probability criterion for global optimum, i.e. Eq. (5), was coded. The program generated a series of uniformly distributed random points as initial searching points, and then SQP was applied to find the optimum based on each initial points. If the probability exceeds a preset value (99.99%, in this study) according to Eq. (5), the global optimum is considered found. Otherwise, next random initial point is generated and SQP executed again. Table 6 shows the initial points and their corresponding optimal CF values and Fig. 1 shows the evolution of all optimal values graphically. From Table 6, there are six different local optimal CF values (from 1.0131 to 1.2944) found from 28 randomly generated

Table 5
The coefficients of probiotic growth rate model and hardness model of probiotic dairy tofu after 12-h fermentation

Coefficient \Y	GR[a] (AC)[b]	GR (B)[c]	Hardness
$ß_0$	0.10	−0.066	−134.81
$ß_1$	−0.098	-4.557×10^{-3}	503.52
$ß_2$	0.046	−0.048	208.61
$ß_3$	−0.013	−0.033	4.26
$ß_4$	9.645×10^{-3}	0.030	7.68
$ß_{11}$	0.037	0.016	−354.42
$ß_{22}$	−0.021	0.027	−107.43
$ß_{33}$	4.007×10^{-3}	5.176×10^{-3}	−2.27
$ß_{44}$	-3.306×10^{-4}	-1.053×10^{-3}	0.31
$ß_{12}$	−0.030	-2.393×10^{-3}	−86.11
$ß_{13}$	5.871×10^{-3}	7.610×10^{-3}	42.58
$ß_{14}$	2.021×10^{-3}	-1.537×10^{-3}	−34.46
$ß_{23}$	-4.855×10^{-3}	-9.272×10^{-3}	−1.47
$ß_{24}$	2.048×10^{-3}	8.749×10^{-4}	−14.65
$ß_{34}$	-1.902×10^{-4}	1.305×10^{-3}	−0.86
$ß_{111}$	0	0	0
$ß_{222}$	0	0	0
$ß_{333}$	0	0	0
$ß_{444}$	0	0	0
$ß_{112}$	0	0	32.61
$ß_{113}$	0	0	−39.66
$ß_{114}$	0	0	26.78
$ß_{122}$	0	0	27.32
$ß_{133}$	0	0	2.75
$ß_{144}$	0	0	0
$ß_{223}$	0	0	−6.03
$ß_{224}$	0	0	7.80
$ß_{233}$	0	0	2.50
$ß_{244}$	0	0	0
$ß_{334}$	0	0	0
$ß_{344}$	0	0	0
$ß_{123}$	0	0	0
$ß_{124}$	0	0	0
$ß_{134}$	0	0	0
$ß_{234}$	0	0	0

[a] GR: growth rate.
[b] AC: *L. acidophilus* + *L. casei*.
[c] B: *B. longum* + *B. bifidum*.

initial points. The rightmost column of Table 6 shows the probability of an optimal CF value being the global one. Among those local optimal values, the global optimal CF value was 1.2944 with 99.99% certainty. The optimal CF value corresponds to 0.1463 of growth rate for *Lactobacillus* spp., 0.1575 of growth rate for *Bifidobacteria* spp. and 94.15 of the product's hardness. There were 11 sets among 28 sets attained the highest optimal CF value (1.2944) with optimal points $X_1 = 1$, $X_2 = 0$, $X_3 = 3$ and $X_4 = 18$. The optimal conditions for manufacturing probiotic dairy tofu were 18% of skim milk blended with 1% of GDL, 0% of peptides and 3% of IMO.

To further depict the global optimization results, 3-D response surface plots were created by fixing two of the four factors. Fig. 2 shows four local maxima including the global one in a CF response function that was produced by setting $X_1 = 1$ and $X_4 = 18$ while varying X_2 and X_3 within their boundaries. Fig. 3 shows the other two local maxima found during the optimization process by fixing $X_3 = 0$ and $X_4 = 18$ while changing X_1 and X_2. The optimization results clearly show that whether the global optimum could be found depends on the initial searching points for our response surface models. To reassure the global optimum that was found was indeed the global one, GAs were also applied on the CF response surface model.

4.2.2. Genetic Algorithms

The composite function was optimized using the SGA and MGA. Fig. 4 shows the evolution curves in searching for the global optimum. The composite function value increased in accordance with the number of function evaluations and reached the maximum value on the both curves. The maximum CF value provided the optimal processing conditions for the probiotic dairy tofu. The number of function evaluations in Eq. (8) represents the efficiency of the algorithms. A smaller number indicates a greater efficiency.

$$N_e = N_g \times N_p \tag{8}$$

where N_e, N_g and N_p represent, respectively, the numbers of total function evaluations, the generations and the population size.

Both searching procedures were allowed to continue to reach the maximum number of function evaluations, i.e. 5000, even though there had been no significant changes in CF values after 3750 function evaluations. In Fig. 4, both SGA and MGA produced fast increasing CF values during the early stage of the optimization processes, which are typical for GAs. For 900 function evaluations, the composite function by SGA has been increased from 1.1446 to 1.2834, compared to 1.0298 to 1.2928 for MGA. The same maximal value (CF value = 1.2944) was obtained in 4600 and 3340 function evaluations for SGA and MGA, respectively. The MGA converged more rapidly to the optimal value than did the SGA. The essences of MGA are the lack of mutations and the mechanism of restarts. Due to these features, the algorithm converges faster to the global maximum.

Population size also affected the results (Fig. 4). An optimal value was obtained at the 92nd generation, i.e. 4600/50 = 92 from Eq. (6), for the SGA curve. For the MGA curve, comparing to the SGA curve, an optimal value was obtained at the 334th generation, i.e. 3340/10 = 334. This means the highest fitness value could be obtained at earlier generations for increasing population size because of the variety of chromosomes. Moriyama and Shimizu (1996) also drew a similar conclusion and indicated that large population size could decrease the generation to reach the highest fitness value.

The elitist strategy used in this study has been known as an effective way for improving the fitness of

Table 6
The randomly generated, initial searching points and optimal CF values found by SQP

Set no.	Initial searching point				Optimal point				Optimal CF value	Probability
	X_1[a]	X_2[b]	X_3[c]	X_4[d]	X_1	X_2	X_3	X_4		
1	0.6911	1.0511	1.0385	12.0752	1	0	0	18	1.2381	0.6667
2	0.8479	1.2047	0.5282	13.8055	1	1.5	0	18	1.2403	0.7000
3	0.3985	0.7750	0.2037	17.7905	0.3	0	0	18	1.1485	0.7143
4	0.6904	0.7540	0.9282	12.6125	1	0	0	18	1.2381	0.7222
5	0.4438	1.1321	1.0043	15.5761	0.3	1.5	0	18	1.1779	0.7273
6	0.9023	0.3572	1.1285	14.8139	1	0	0	18	1.2381	0.7308
7	0.4801	1.4146	2.8566	16.304	1	1.5	3	18	1.0131	0.7333
8	0.4943	0.1329	2.158	17.1532	1	0	3	18	1.2944	0.7353
9	0.5017	0.8145	2.338	13.1136	1	0	3	18	1.2944	0.8762
10	0.8143	1.3552	1.853	14.7742	1	1.5	0	18	1.2403	0.8759
11	0.6479	1.2920	1.9477	17.4198	1	1.5	0	18	1.2403	0.8758
12	0.3383	0.9472	2.2688	12.1326	1	0	3	18	1.2944	0.9435
13	0.4339	0.3935	0.4435	16.5624	0.3	0	0	18	1.1485	0.9430
14	0.8072	0.5685	1.7984	16.7751	1	0	3	18	1.2944	0.9747
15	0.9816	0.5060	2.6958	13.8831	1	0	3	18	1.2944	0.9891
16	0.7931	0.4719	0.5158	13.415	1	0	0	18	1.2381	0.9888
17	0.8611	1.4207	2.4568	14.9667	1	1.5	3	18	1.0131	0.9886
18	0.4444	0.8028	0.2079	16.1115	0.3	0	0	18	1.1485	0.9883
19	0.8409	1.0529	2.8671	17.6678	1	0	3	18	1.2944	0.9950
20	0.9109	1.3126	0.952	14.6359	1	1.5	0	18	1.2403	0.9948
21	0.8652	1.4794	0.0156	14.9846	1	1.5	0	18	1.2403	0.9947
22	0.8296	1.3280	2.2798	12.5917	1	0	3	18	1.2944	0.9977
23	0.9332	0.6073	0.926	15.3266	1	0	0	18	1.2381	0.9977
24	0.5947	0.9407	2.1458	14.6142	1	0	3	18	1.2944	0.9990
24	0.7609	0.5782	0.2428	13.0876	1	0	0	18	1.2381	0.9990
26	0.3009	1.2718	2.5376	12.9229	1	0	3	18	1.2944	0.9996
27	0.9874	0.7903	2.1551	17.6024	1	0	3	18	1.2944	0.9998
28	0.5886	1.2111	2.6112	12.8176	1	0	3	18	1.2944	0.9999

[a] X_1: GDL%.
[b] X_2: Peptide %.
[c] X_3: IMO%.
[d] X_4: Skim milk powder %.

chromosomes because a chromosome with maximum fitness is compulsorily remained for next generation (Morimoto, Purwanto, Suzuki, & Hashimoto, 1997).

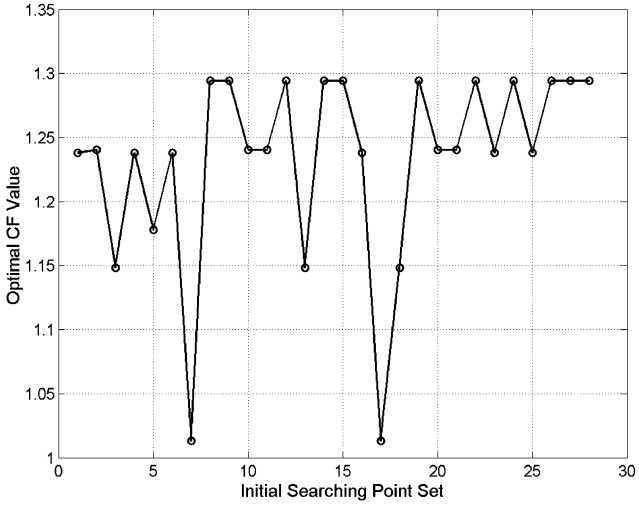

Fig. 1. Optimum CF values for randomly generated initial searching points when using SQP.

4.2.3. Comparison between GAs and SQP

Comparing the optimization results, the SQP and GAs all produced the global optimum in this research. Both GAs yielded comparable results with MGA being

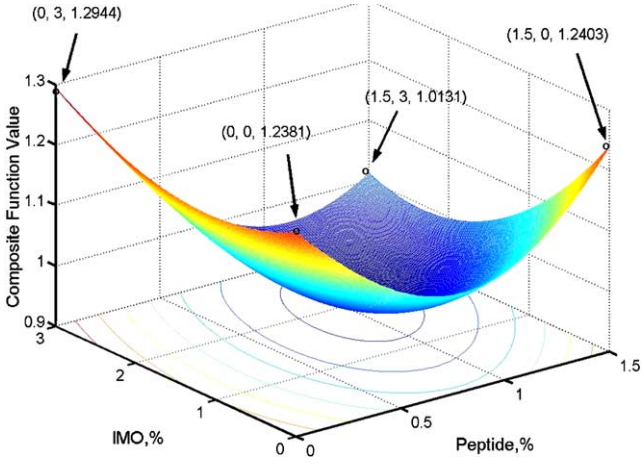

Fig. 2. A response surface plot under the conditions of constant GDL (1%) and skim milk (18%).

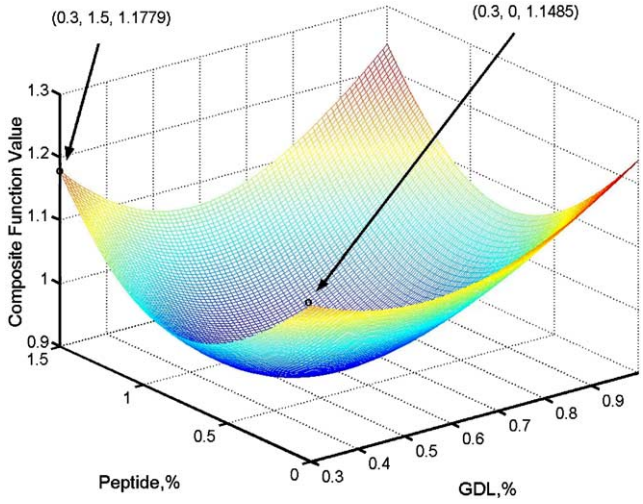

Fig. 3. A response surface plot under the conditions of constant IMO (0%) and skim milk (18%).

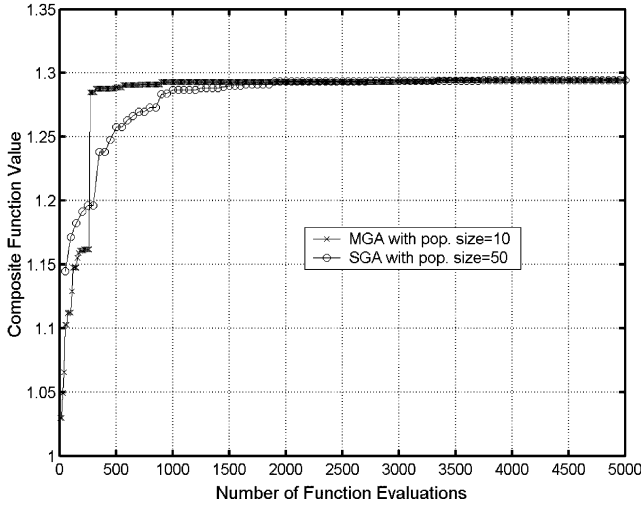

Fig. 4. Evolution curves of the two Genetic Algorithms for searching the optimal processing conditions for the probiotic dairy tofu.

slightly more efficient than SGA. Although the SQP's searching performance is dictated by the initial searching points in our study, the modified SQP with the multi-start capability reached the global optimum with 99.99% certainty in 28 tries. The SQP was obviously the most efficient method of all.

4.2.4. Effects of optimized factors on responses

The optimal manufacturing conditions were found to be 1% of GDL (upper limit), 3% of IMO (upper limit), 0% of peptides (lower limit) and 18% of milk concentration (upper limit). Addition of skim milk powder can increase the total solid of milk and improve the hardness of dairy tofu. GDL is allowed for use in human food as a coagulant and a pH control agent. Increasing the GDL level can raise the hardness of probiotic dairy tofu. Therefore, both milk concentration and GDL in their upper limits were suggested by the optimization methods. As for IMO and peptides, both are growth promoters for probiotics. IMO can stimulate lactic microflora as well as facilitate the elevated product of butyrate (Fooks, Fuller, & Gibson, 1999). Consequently, the highest level of IMO was recommended by the optimization procedures. Peptides have been proved to be able to improve the viability of bifidobacteria (Dave & Shah, 1997). According to our preliminary tests, peptides did increase the growth rate of bifidobacteria, but decreased the hardness of dairy tofu. The composite function (Eq. (4)) is composed of all three responses. If the addition of a certain factor increases one response but decreases another, a compromise will be made to achieve a higher composite function value during optimization. This is why peptides attained the value of the lower limit in our results. The optimal conditions are the results of the interactions among the four factors and the combined effects of all three responses.

4.3. Experimental verification

The optimal producing conditions were suggested by the MGA, SGA and SQP, and were verified by additional independent experiments. The optimal manufacturing conditions were obtained as those of maximum GDL (1%), IMO (3%) as well as milk concentrations (18%), and in combination with a minimal peptides level (0%). The three responses, the growth rates of *Lactobacillus* spp. and *Bifidobacteria* spp. and the hardness of the dairy tofu, and the composite function value produced by verification experiments were all very close to the predicted values with no apparent significant differences being demonstrated between the two sets ($P > 0.05$).

5. Conclusion

The two-stage effort of obtaining a surface model using RSM, and optimizing this model using GAs or SQP techniques has been demonstrated to represent an effective approach. Both SQP and GAs techniques are able to determine the optimal conditions for manufacturing the probiotic dairy tofu. The conditions were 1% of GDL, 0% of peptides level, 3% of IMO and 18% of milk concentrations, and they were confirmed by verification experiments. Among the SQP and two GAs employed, the SQP, modified with the multi-start capability, is the most efficient one.

Acknowledgment

The authors wish to thank the National Science Council of Taiwan, ROC (NSC90-2313-B254-001) for their support of this research.

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