Optimization of the Growth Rate of Probiotics in Fermented Milk Using Genetic Algorithms and Sequential Quadratic Programming Techniques

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ABSTRACT : Prebiotics (peptides, N-acetyglucoamine, fructo-oligosaccharides, isomalto-oligosaccharides and galacto-oligosaccharides) were added to skim milk in order to improve the growth rate of contained *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium longum* and *Bifidobacterium bifidum*. The purpose of this research was to study the potential synergy between probiotics and prebiotics when present in milk, and to apply modern optimization techniques to obtain optimal design and performance for the growth rate of the probiotics using a response surface-modeling technique. 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 a genetic algorithm and sequential quadratic programming approach to obtain the maximum growth rate of the probiotics. The results showed that the quadratic models appeared to have the most accurate response surface fit. Both SQP and GA were able to identify the optimal combination of prebiotics to stimulate the growth of probiotics in milk. Comparing both methods, SQP appeared to be more efficient than GA at such a task. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 6 : 894-902)***

Key Words : Probiotics, Optimization, Genetic Algorithms, Sequential Quadratic Programming

INTRODUCTION

In recent years, there has been a worldwide increase in interest in the addition of intestinal bacterial species (*Bifidobacterium* spp. *Lactobacillus acidophilus*, *L. casei*) to fermented milks. 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 the intestinal tract of consumers, but also due to the expanding variety of products that can be formulated with probiotics bacteria (Liu et al., 2002). It has been recommended that foods containing probiotics bacteria should contain at least 10^7 live microorganisms per gram at the time of consumption, in order for the bacterial level to maximally benefit the consumer (Ishibashi and Shimamura, 1993; Playne, 1994; Desmond et al., 2002).

A number of substances are well known to improve the growth of probiotic bacteria, eg the supplementation of milk with a combination of casitone, casein hydrolysate and fructose stimulates the growth of *L. acidophilus* (Saxena et al., 1994). Such stimulation in growth is suggested to arise due to an enhanced availability of simple sugars, mainly glucose and fructose, and minerals which have been demonstrated to be growth promoters of *L. acidophilus* (Lourens-Hattingh and Viljoen, 2001). Dave and Shah (1998) investigated the effect of cysteine, acid hydrolysates, tryptone, whey protein concentrate and whey protein upon the viability of yogurt and probiotic bacteria in yogurt, they concluding that the addition of each of these supplements, except whey powder, improved the viability of bifidobacteria to a variable extent in the yogurt made with ABT (*L. acidophilus*, bifidobacteria, *S. thermophilus*) starter culture.

In addition to the probiotic approach of directly introducing live bacteria into the colon through dietary supplementation (Mattila-Sandholm et al., 2002), another approach to increase the number of beneficial bacteria such as bifidobacteria in the realm of intestinal microbiota is through the use of prebiotics (Holzapfel and Schillinger, 2002). Prebiotics are non-digestible dietary components that pass through to the colon and are not affected by such passage, and, when present in the colon, they selectively stimulate the proliferation and /or activity of populations of desirable bacteria in the gut (Holzapfel and Schillinger, 2002). Due to the potential synergy between probiotics and prebiotics, foods containing a combination of these ingredients are often referred to as synbiotics, although synbiotics (Mattila-Sandholm et al., 2002) have not been intensively studied to date. Bielecka et al. (2002) confirmed the appropriate selection of synbiotics and demonstrated their higher effectiveness as compared to probiotics.

The purpose of this research was to study the potential synergy that exists between probiotics and prebiotics in milk and to attempt to apply modern optimization techniques to obtain optimal processing conditions and performance of the growth rate of selected probiotics by a

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Table 1. Nature and coded variables and levels

Independent variable	Symbol	Level	
		Coded	Nature
Peptides conc. (%)	X_1	-1	0.00
		0	0.75
		+1	1.50
NAG conc. (%)	X_2	-1	0.00
		0	0.50
		+1	1.00
FOS conc. (%)	X_3	-1	0.00
		0	1.50
		+1	3.00
IMO conc. (%)	X_4	-1	0.00
		0	1.50
		+1	3.00
GOS conc. (%)	X_5	-1	0.00
		0	1.50
		+1	3.00

process of response surface modeling. The end-point was to establish a new optimization method to improve the growth rate of probiotics within dairy products.

MATERIAL AND METHODS

Preparation of drinking yogurt

Prebiotics were mixed with milk (12% total solids; Anchor Foods, New Zealand) in order to attempt to improve the growth rate of *L. acidophilus*, *L. casei*, *B. bifidum* and *B. longum*. According to Mitsuoka *et al.* (1987) and our screening test, the growth rate of *L. acidophilus*, *L. casei*, *B. bifidum* and *B. longum* were affected by five prebiotics, these including: peptides (0.0-1.5%, Cheng-Fung, Co., Taiwan), fructooligosaccharides (FOS, 0.0-3.0%, Cheng-Fung, Co., Taiwan), isomaltooligosaccharides (IMO, 0.0-3.0%, Ying-Yu Co., Taiwan), gala-tooligosaccharides (GOS, 0.0-3.0%, Cheng-Fung, Co, Taiwan) and N-acetylglucosamine (NAG, 0.0-1.0%, Sigma, Germany).

After heat treatment at 85°C for 30 minutes, samples were inoculated with 1% *L. acidophilus*, 1% *L. casei*, 1% *B. bifidum* and 1% *B. longum* suspensions and fermented for 10 h at 37°C.

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, R.O.C. Lactobacilli MRS (deMan, Rogosa and Sharp, Merk, Germany) and Lithium propionate MRS agar (LP-MRS, Merk, Germany) were used as the selective media for *Lactobucillus* spp. and *Bifidobacteria* spp., respectively (Lapierre et al., 1992).

Activity determination

One milliliter samples of fermented milk were decimally diluted into sterile peptone water (0.1%) following which 0.1ml aliquots of the dilutions plated over the culture media. Plates of MRS agar were incubated aerobically for 72 h at 37°C so as to select *Lactobacillus* spp. and inhibit Bifidobacteria activity. Plates of LP-MRS agar were incubated anaerobically (72 h at 37°C; GasPak System-Oxoid, Basingstoke, Hampshire, England) for Bifidobacteria. Plates of LP-MGL agar were incubated aerobically for 72 h at 37°C for *L. casei subsp. rhamnosus*. The various bacterial populations, scored in colony-forming units (CFU), and the characteristics of the colonies were subsequently recorded for each medium.

For the determination of the viabilities of the probiotics, the populations of *Lactobacillus spp.*, *L. casei*, and *bifidobacteria spp.* were measured as growth rates. The specific growth rate (GR) corresponding to each culture was calculated using the following equation:

$$GR = (\log X_1 - \log X_2)/(t_1 - t_2) \quad (1)$$

where X_1 and X_2 are the CFU at time t_1 (fermentation at 0 h) and t_2 (fermentation at 10 h).

Modeling and optimization

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 a genetic algorithm and a sequential quadratic programming (SQP) approach in order to obtain a means by which the maximum growth rate of probiotics could be guaranteed.

Response surface model mathematical formulation : The main advantage of RSM is the reduced number of experimental trials needed in order to effectively evaluate multiple parameters and their interactions (Porretta et al., 1995; Lee et al., 2000). A Central Composite Design (CCD) with 6 replicates at the centerpoint was used. The CCD is the most frequently used response surface method design and the design descriptions and analyses are done best with coded factors (Myers and Montgomery, 1995). The coding schemes set -1 as the lower level of a factor, +1 as the upper level, and 0 as the middle level. A CCD can be broken down into three groups of design points (Myers and Montgomery, 1995):

(i). Two-level factorial or fractional factorial design ; The two-level factorial part of the design consists of all possible combinations of the +1 and –1 levels of the coded factors. For a three-factor case, there are 2^3=8 design points: (-1, -1, -1), (1, -1, -1,), (-1, 1, -1), (-1, -1, 1), (1, 1, -1), (1, -1, 1), (-1, 1, 1) and (1, 1, 1).

(ii). Axial (star) points ; Axial (star) points have all the factors set to 0, the midpoint, except for one factor set at a value plus or minus alpha (α). For a three-factor problem, the star points are: (-α, 0, 0), (α, 0, 0), (0, α, 0), (0, -α, 0), (0, 0, α) and (0, 0, -α). There are many practical situations in which the scientist specifies ranges on the design factors, and these ranges are strict. In these cases, the α is set to 1 and the design is called the face center cube.

(iii). Center points ; Data from the center points provides estimates of pure error and estimates of curvature.

For our studies with five factors, the total number of runs is equal to 48, 2^5 (two-level factorial)+2×5(axial points)+6(center points)=48. The coded and uncoded variables and their respective level are shown in Table 1. The RSM procedure of the Design-Expert® software package (Stat-Ease, Inc., USA, 2000) was used to fit the experimental data to polynomial equations of order 1 through 3 to obtain coefficients using the regression techniques. The following relationship achieved this.

$$Y_i = f_i(X_1, X_2, X_3, X_4, X_5) + \varepsilon_i \; i = 1,2,3 \qquad (1)$$

where Y_1, Y_2, Y_3 were the observed growth rate of *Lactobacillus spp*, *Bifidobacterium spp.* and *Lactobacillus casein*, respectively. f_1, f_2, f_3 represented the modeled response surfaces. X_1, X_2, X_3, X_4, X_5 defined as natural variables, were the concentrations of peptides, fructooligosaccharides, isomaltooligosaccharides, galatooligosaccharides and N-acetylglucosamine, respectively. ε_1, ε_2, ε_3 were the errors in each model, the errors were assumed to be normally distributed with mean zero and constnat variance. With RSM, it is convenient to transform the natural variables to coded variables ξ_1, ξ_2, ξ_3, ξ_4, ξ_5, where the coded variables are defined as dimensionless, with mean zero and the same spread or standard deviation:

$$Y_i = f_i(\xi_1, \xi_2, \xi_3, \xi_4, \xi_5) + \varepsilon_i \; i = 1,2,3 \qquad (2)$$

Genetic algorithms (GAs) : The GAs provide a very flexible framework and recently have been regarded as not only a global optimization method but also a multi-objective optimization method in various areas. Generally, the algorithms can be described as the following steps (Goldberg, 1989; Mitchell, 1996): (i). Start with a randomly generated population of n l -bit chromosomes. (ii). Calculate the fitness $f(x)$ of each chromosome x in the population. (iii). Repeat the following steps until n offspring have been created: a. Select a pair of a parent chromosomes from the current population, the probability of selection being an increasing function of fitness. b. With crossover rate, cross over the pair at a randomly chosen point to form two offspring. c. Mutate the two offspring at a prescribed mutation rate, and place the resulting chromosomes in the new population. (iv). Replace the current population with the new population. Each iteration of this process is called a generation. The above procedure is called the simple GA (SGA).

Table 2. The parameters relevant to a Simple Genetic Algorithm (SGA) and a Micro Genetic Algorithm (MGA)

Parameter	SGA	MGA
Population size	100	10
No of bits	20	20
Mutation rate (%)	0.02	0
Crossover rate (%)	0.5	0.5
Max. generation number	100	500

Besides simple GA, another variation of GAs, micro GA (MGA), was also employed in this paper. The essence of the MGA is the lack of mutations and the presence of restarts. Due to this feature, the algorithm converges rapidly to a local or global maximum (Nikitas et al., 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 composite function fitness is retained and the rest *N*-1 chromosomes (*N* is the total number of chromosomes in one generation) are replaced by the same number of randomly generated new ones.

In order to use the GAs, a chromosome was formed by 5 different prebiotics: peptides, N-acetyglucoamine, fructo-oligosaccharides, isomalto-oligosaccharides and galacto-oligosaccharides, which were all coded as 20-bit binary strings. Table 2 shows all the parameters of the SGA and MGA used in the paper.

In order to search a solution that maximized multiple responses using GAs, a composite function fitness was defined as the following:

Composite Function Fitness

$$(\text{CFF}) = \left(f_1 \times f_2 \times f_3\right)^{1/3} \qquad (3)$$

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

Sequential quadratic programming : A quadratic programming problem is an optimization problem involving a quadratic objective function and linear constraints. The Sequential Quadratic Programming (SQP) method represents state-of-the-art in nonlinear programming methods (The Math Works, 2000) and can be used to iteratively solve quadratic programming problems. 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. Belegundu and Chandrupatla (1999) and The Math Works (2000), and will not be elaborated here. The SQP procedure implemented by the Matlab software package (The Math Works, 2000) was

Table 3. Analysis of variance for the variables as linear, quadratic and cubic parameters and their interactions in a response variable model

Source	AC[a]		B[b]		C[c]	
	Sum of squares	Prob.>F	Sum of squares	Prob.>F	Sum of squares	Prob.> F
(a) Model analysis[a]						
Mean	2.78		2.56		2.90	
Blocks	1.96×10^{-3}		6.46×10^{-3}		2.78	
Linear	0.03	<0.01**	0.02	<0.01**	0.04	<0.01**
Quadratic	0.02	<0.01**	0.02	0.01*	0.02	<0.01**
Cubic	4.42×10^{-3}	0.47	5.26×10^{-3}	0.47	4.93×10^{-3}	0.08
Residual	2.44×10^{-3}		2.90×10^{-3}		1.16×10^{-3}	
Total	2.84		2.61		2.97	
(b) Lack-of-fit test results[b]						
Linear	0.03	0.20	0.02	0.45	0.02	0.07
Quadratic	5.57×10^{-3}	0.64	6.29×10^{-3}	0.76	5.49×10^{-3}	0.29
Cubic	1.15×10^{-3}	0.65	1.03×10^{-3}	0.80	5.65×10^{-3}	0.62
Pure error	1.29×10^{-3}		1.87×10^{-3}		5.91×10^{-3}	
(c) R-square analysis[c]						
Linear	0.53	0.04	0.50	0.03	0.61	0.04
Quadratic	0.89	0.03	0.82	0.03	0.90	0.03
Cubic	0.96	0.11	0.94	0.09	0.98	0.05

* Significant at 5% level; ** significant at 1% level

[a] AC: L. acidophilus+L. casei.

[b] B: Bifidobacterium.

employed to attempt to optimize the growth rate of probiotics that were formulated, via RSM, as a quadratic function of five independent variables bounded by presented upper and lower limits.

RESULTS AND DISCUSSION

Response surface modeling

The present work has developed a prediction model for the growth rate of probiotics by using RSM, this model also determining the effect of prebiotics on the viability of probiotics when in combination.

Fitting the model: Five prebiotics (peptides, fructo-oligosaccharides, isomaltooligosaccharides, galacto-oligosaccharides and N-acetylglucosamine), which were defined as the designed variable, were mixed with milk in order to attempt to improve the growth rate of *L. acidophilus*, *L. casei*, *B. bifidum* and *B. longum*, which were defined as the responses. The responses as linear, quadratic and cubic functions of the variables were tested for adequacy and fitness using analysis of variance (ANOVA). The selections of adequacy models (table 3) were using model analysis, lack-of fit test and R-square analysis as out line by Lee et al. (2000) and Weng et al. (2001). table 3(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 Tests" (Table 3b) compares the residual error to the 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

Table 4. Regression coefficients for the second-order polynomials

Coefficient\Y	GR[a] (AC)[b]	GR (B)[c]	GR (C)[d]
β_0	0.17	0.17	0.2
β_1	0.14	0.095	0.15
β_2	0.054	0.048	3.809E-03
β_3	-0.047	-0.028	-0.038
β_4	0.02	0.012	9.767E-05
β_5	0.018	0.022	3.517E-03
β_{11}	-0.064	-0.05	-0.06
β_{22}	-5.258E-03	-0.01	8.265E-03
β_{33}	5.389E-03	5.496E-03	5.624E-03
β_{44}	-2.724E-03	-7.048E-04	3.838E-04
β_{55}	-4.088E-03	-4.962E-03	-2.591E-03
β_{12}	0.015	0.015	8.093E-03
β_{13}	0.01	6.517E-03	1.897E-03
β_{14}	-2.444E-03	5.363E-03	1.561E-03
β_{15}	-3.504E-03	2.507E-03	-2.987E-03
β_{23}	1.798E-03	6.297E-03	1.597E-03
β_{24}	-0.025	-0.023	-0.014
β_{25}	-0.017	-0.016	-8.528E-03
β_{34}	5.539E-03	5.314E-04	4.09E-03
β_{35}	0.01	4.632E-03	0.01
β_{45}	-2.876E-03	-4.404E-03	1.844E-04

[1] β_0 represents intercept and β_1, β_2, β_3, β_4 and β_5 represent the five variables of peptides, fructooligosaccharides, isomaltooligosaccharides, galatooligosaccharides and N-acetylglucosamine. β_{11}, β_{22} , β_{33}, β_{44} and β_{55} represent the respective square terms. β_{12}, β_{23}..., β_{45} are the interaction terms.

[a] GR: Growth rate

[b] AC: L. acidophilus + L. casei.

[c] B: Bifidobacterium.

[d] C: L. casei.

selected. ANOVA showed that the quadratic models appeared to be the most accurate for all three responses, with no significant lack of fit (table 3). Second-order polynomial equations (4) were fitted to the experimental data using the Design Expert procedure:

$$f_k = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad k=1, 2, 3 \qquad (4)$$

where f_k were the three responses and β_0, β_i, β_{ii}, and β_{ij} were constant coefficients and X_i were the uncoded independent variables. The regression coefficients for the statistically significant models are given in Table 4.

The effect of prebiotics upon the viability of probiotics : Estimation of the overall influence of the five prebiotics affecting the growth rate of probiotics using ANOVA indicates that all variables were significant in this regard ($p<0.05$). The relationships between the variables and the responses were also investigated by examining a series of 3-D plots generated by holding constant some of the variables of the CFF, which was composed of three second-order polynomial equations (eq.4).

As Figure 1a indicates, the relative composite function fitness increases with increasing NGA when the concentration of oligosaccharides is zero, however, Figures 1b and 1c reflect differing results. The composite function fitness increases with increasing peptide and decreasing NGA concentration, results indicating that NGA was able to stimulate the growth of probiotics only when oligosaccharides were not present in the solution. A number of earlier studies have investigated the effects of peptides upon human gut bacteria (Mitsuoka et al., 1987; Dave and

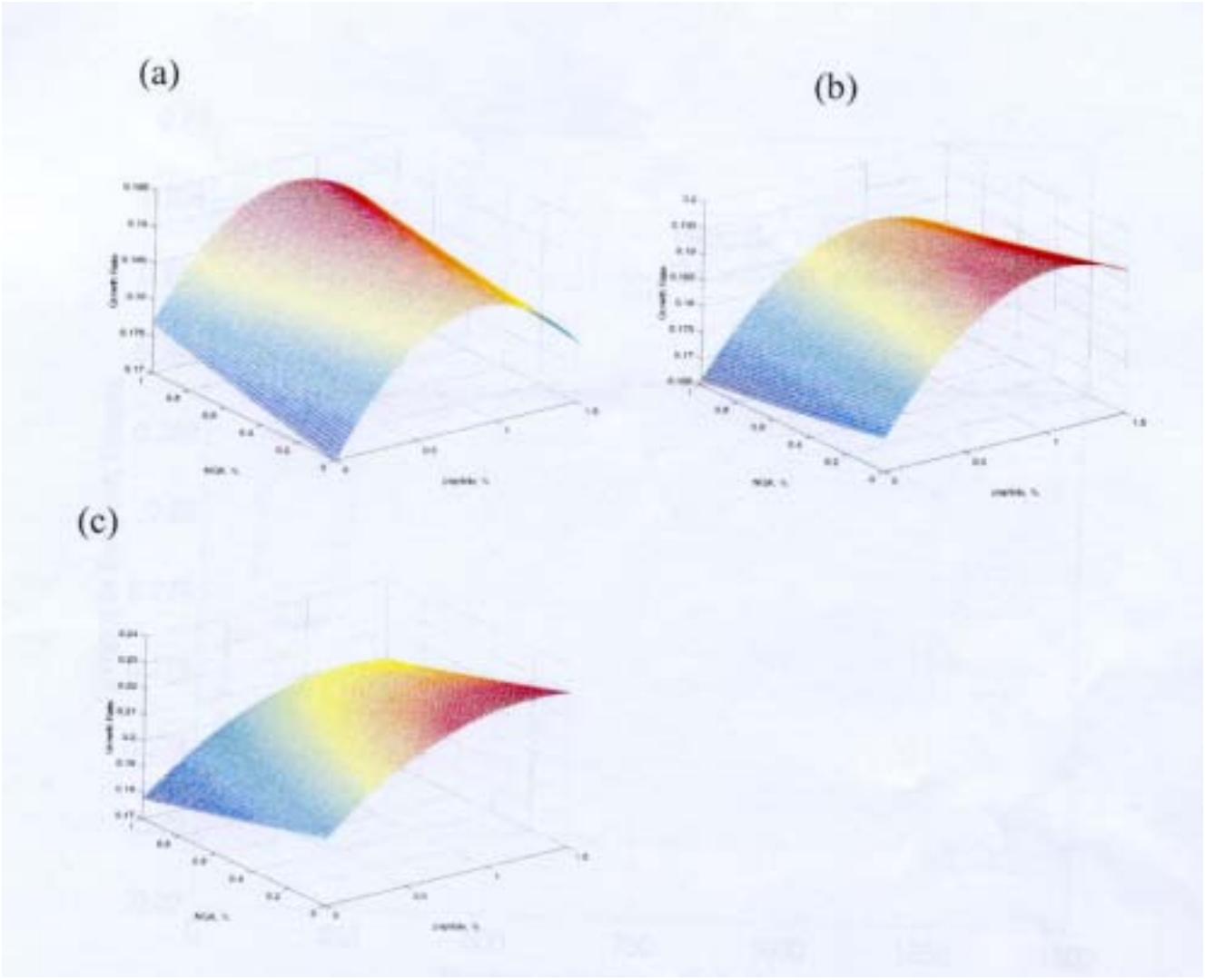

Figure 1. Response surface plots of composite function fitness showing effects of peptides and NGA under the conditions of constant FOS, IMO and GOS: (a) FOS=0.0%, IMO=0.0%, GOS=0.0%, (b) FOS=1.5%, IMO=1.5%, GOS=1.5%, (c) FOS=3.0%, IMO=3.0%, GOS=3.0%.

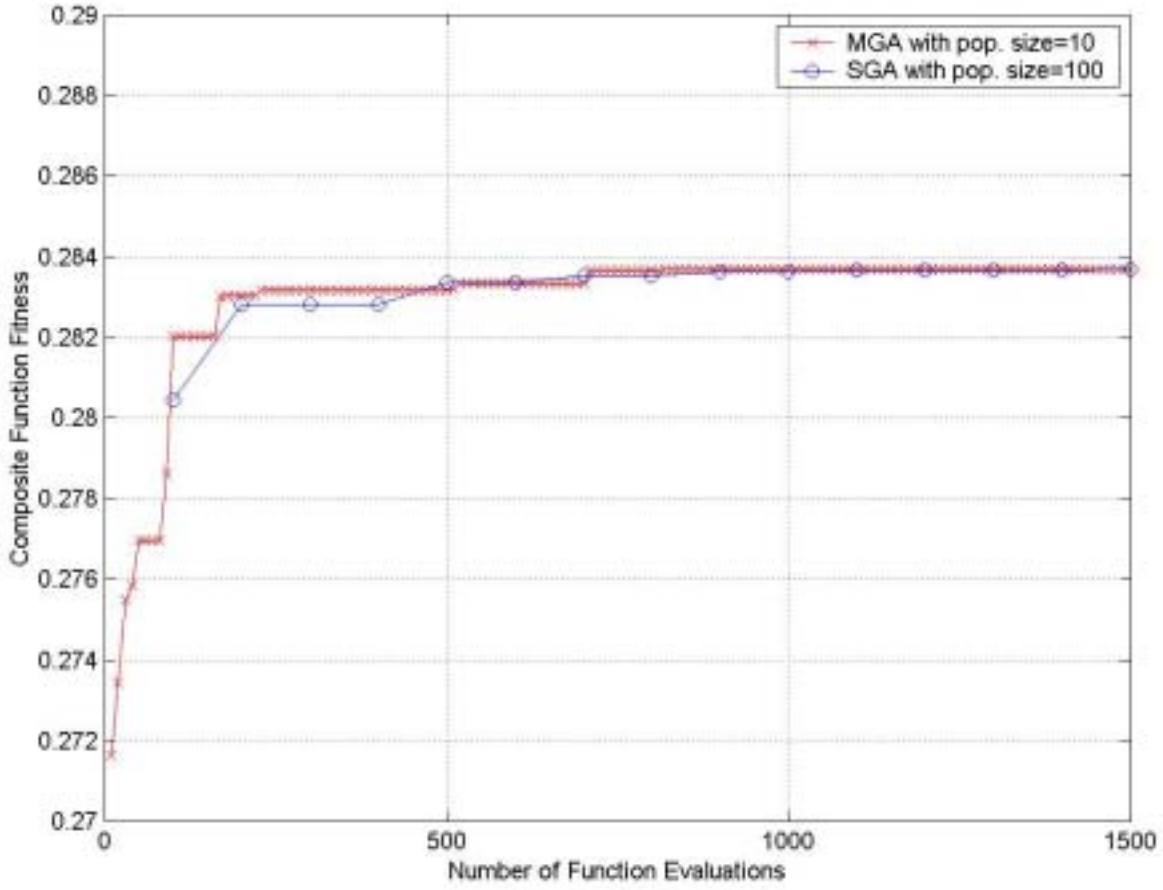

Figure 2. Evolution curves in searching for an optimal value under different search procedures of the genetic algorithm.

Shah, 1998; Lourens-Hattingh and Viljoen, 2001). The nitrogen sources here, in the form of various peptides and amino acids, probably acted by improving the viability of the bifidobacteria present in the gut (Lourens-Hattingh and Viljoen, 2001). Dave & Shah (1998) investigated the effects of peptides and confirmed that the addition of peptides improved the viability of Bifidobacteria, to a variable extent, in yogurt. Mitsuoka et al. (1987) suggested that the function of peptide in this context was to act as a Bifidobacteria growth promoter, this being confirmed by a subsequent *in-vitro* test.

Varying the quantity of FOS, IMO and GOS from zero to 3% significantly affected the growth rate of probiotics (Figures 1a, 1b, 1c), such a growth rate increasing as the FOS, IMO and GOS levels were increased. A number of non-digestible oligosaccharides have now been developed, for which there exists some evidence of a prebiotic effect, these including fructo-oligosaccharide, gluco-oligosaccharides and isomalto-oligosaccharides (Hayakawa et al., 1990; Gibson et al., 1999). In general, the feeding of FOS increases the numbers of *Bifidobacteria* spp. and *Lactobacilli* spp., increases SCFA concentrations and reduces levels of clostridia, fusobacteria and bacterioides, and also pH (Hayakawa et al., 1990; Gibson et al., 1999). The estimated intake doses of FOS which have been suggested to elicit a bifidogenic effect in human studies ranges from 4 to 15 g/day (Djouzi and Andrieux, 1998). The current study demonstrates that addition of 3% FOS and 1.5% peptide in milk are able to stimulate the growth of Lactobacilli and Bifidobacteria during fermentation. Isomalto-oligosaccharides have been previously revealed to be able to be fermented by bifidobacteria but not by *E. coli* and other bacterial populations. Fooks et al. (1999) have revealed that IMO are very efficient prebiotic agents in that they are able to stimulate lactic microflora as well as facilitating the elevated production of butyrate, which is thought to be a desirable metabolic in the gut. The current study confirms that both GOS and IMO improve the growth rate of probiotics.

Search for an optimal growth rate using genetic algorithm and sequential quadratic programming

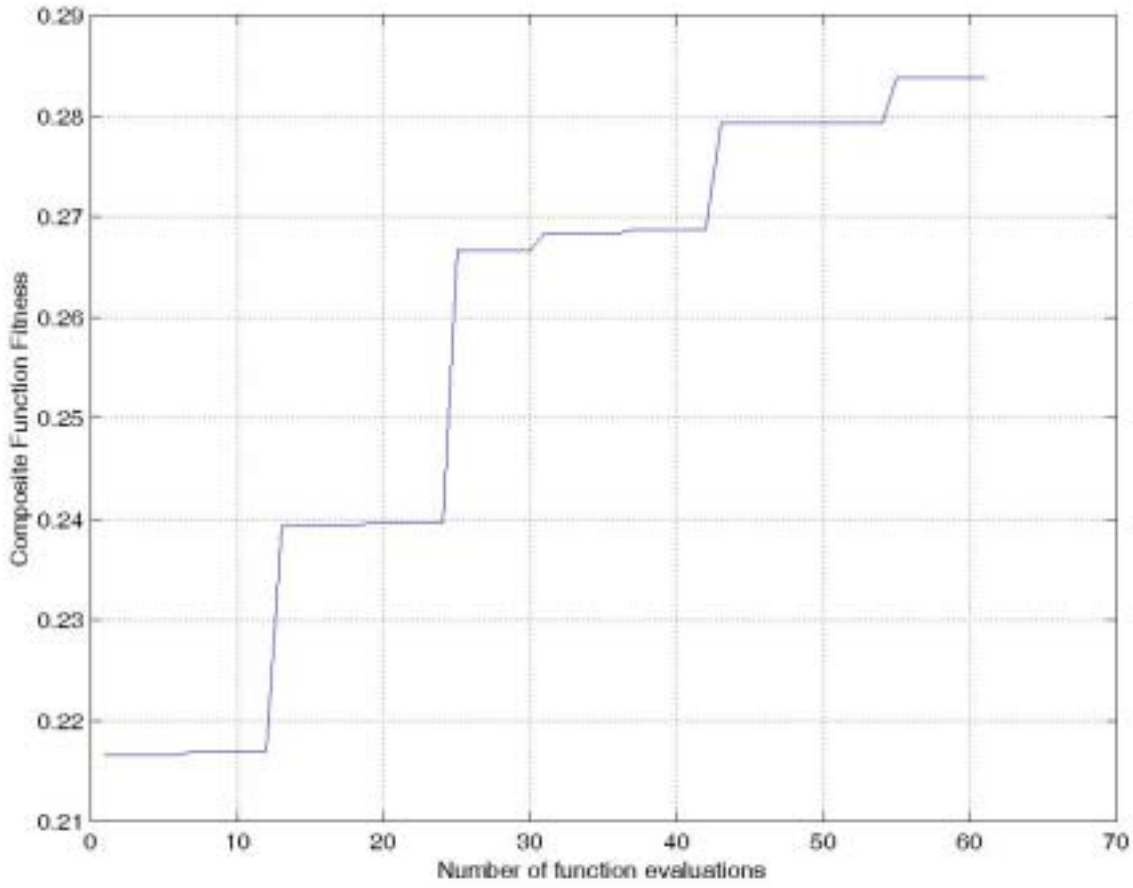

Figure 3. An evolution curve in searching for an optimal value using the sequential quadratic programming technique.

In this research, the CFF was optimized using two methods i.e. GAs and SQP, and the efficiency of the methods were compared.

Genetic algorithms : The CFF was optimized using the GAs. Fig. 2 shows the evolution curves in searching for an optimal value. The CFF increased in accordance to the number of function evaluations and reached the maximum value in the curves of the MGA and SGA. The searching procedure was stopped when the CFF continued to keep the same maximum value with increasing numbers of function evaluations. The chromosomes having the maximum CFF provided the optimal ratios of concentrations of the prebiotics. The number of function evaluations in eq. (5) represents the efficiency of the algorithms. A smaller number indicates a higher efficiency.

$$\text{Number of function evaluations} = \text{Number of generations} \times \text{population size} \quad (5)$$

In figure 2, both methods produced fast increasing CFF during the early stage of optimization process, which is typical for GAs. For 300 function evaluations, the CFF by SGA has been increased from 0.2804 to 0.2828, compared to 0.2802 to 0.2833 for MGA. The same optimal value (CFF=0.2837) was obtained in 1,100 and 720 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 rapidly to maximum (Nikitas et al., 2001) and still maintains the ability to reach the global optimum.

Sequential quadratic programming : The SQP method was used as the basis for implementing the constrained non-linear optimization techniques specified herein. An optimal growth rate was obtained at the 61 function evaluations during the SQP calculation. For 61 function evaluations, the CFF by SQP has been increased from 0.217 to 0.286 (Figure 3), Comparing the CFF results, this study indicated that SQP was more efficient than GA in finding the optimal growth rate. Currently, SQP methods are considered to be the most-efficient methods for solving non-linear

Table 5. (a) The optimum producing conditions (b) The validation of the optimum producing conditions of fermented milk as recommended by GAs and SQP modelling

(a)

Concentration (%)	Peptides	NAG	FOS	IMO	GOS
MGA	1.30	0.00	2.99	3.00	3.00
SGA	1.30	0.00	2.99	3.00	3.00
SQP	1.30	0.00	3.00	3.00	3.00

(b)

	AC[a]		B[b]		C[c]	
Growth rate	Pred[d]	Exp[e]	Pred	Exp	Pred	Exp
MGA	0.33	0.31	0.30	0.28	0.35	0.34
SGA	0.33	0.31	0.30	0.28	0.35	0.34
SQP	0.33	0.31	0.30	0.28	0.35	0.34

[a] AC: L. acidophilus+L. casei.
[b] B: Bifidobacterium.
[c] C: L. casei.
[d] Pred:predict value.
[e] Exp: experimental value.
* $p< 0.05$.
** $p< 0.01$.

programming problems of small to medium size, with a wealth of methods of this kind having been developed (Spellucci, 1998), although it has been suggested that the ongoing search for an optimal method might be stuck in a "local maximum" region and not a global one due to the high dimensionality and irregularities contained within the objective function response (Wang, 1997).

Because the predicted quadratic model in this research is relatively simplistic, the results indicated that both SQP and GA could be effectively utilized to determine the optimal combination of prebiotics to stimulate the growth of probiotics in milk. Comparing both methods, SQP appeared to be much more efficient than GA at this task.

Model verification : The optimal producing conditions were suggested by the MGA, SGA and SQP, and were verified by additional independent experiments (Table 5). These conditions were realized by using 100 mL of skim milk, mixed with the prescribed prebiotics, which were inoculated and fermented for ten hours at 37°C. Table 5 indicate that the resultant optimal ratio of prebiotics, as determined, individually, by the MGA, SGA and SQP algorithms was the same. The maximum growth rate of probiotics was obtained under conditions of maximum peptide concentrations including the peptides FOS, IMO as well as GOS, and in combination with minimal NGA levels. The final responses in a practical case provided a result that was very close to the preditic values with no apparent significant difference being demonstrated between the two ($p>0.05$).

CONCLUSION

The current study suggests that the peptides, IMO, FOS and GOS are able to stimulate the growth of Lactobacilli and Bifidobacteria. These prebiotics may demonstrate the potential for their incorporation into bio-yogurt in order to enhance the number and concentration of probiotics present not only in the colon of the consumer, but also during the process of fermentation of milk. 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 method for this purpose. Both SQP and GAs techniques are able to be used in order to determine the optimal combination of prebiotics necessary to stimulate the growth of probiotics in milk. Comparing both methods, SQP was deemed to be more efficient than GAs at such a task.

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