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Taguchi's Robust Design Method for Optimization of Lysophosphatidic Acid Production in an Open Reactor System

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Abstract The determination of appropriate parameters and parameter conditions is very important for the optimization of production of target materials. Taguchi's method has been used widely as the basis for development trials and optimization during industrial process design. Reaction variables which influence product yield are easily determined and their effects are revealed by just a few reactions, negating the need for extensive experimental investigation. There are usually some factors that are responsible for variations in process characteristics, so called noise factors. Controlling noise factors is very costly and difficult or impossible. Taguchi's experimental design method was examined to determine the control factor's level that is less sensitive to the changes in environmental conditions and other noise factors without control of noise factors. In this study, optimization of lipase-catalyzed production of lysophosphatidic acid (LPA) which has various physiological functions was performed by Taguchi's method. We obtained LPA yields (66.5%) with low variance (5.32) at 400 RPM, molar ratio of 40 : 3 (mmol) (fatty acid : G-3-P), 48 h, and 50°C. Thus, bioactive LPA with a desired fatty acid moiety could be produced with high yields and low variance despite various environmental noise factors.

Key words: Lipase-catalysis, lysophosphatidic acid, Taguchi's robust design method, optimization, open reactor system

Lysophospholipids have been widely studied as new emulsifying agents, pharmaceutical agents and food preservatives [15]. Lysophospholipids also have several physiological functions [1, 2, 10, 12, 17, 19] and mediate several cellular mechanisms. As a result, research into lysophosphatidic acid (LPA) is progressing rapidly. The increasing awareness of the importance of LPA's role in physiological events has also had a great impact on lysophospholipid research. A better understanding of LPA's role in platelet aggregation and pathophysiology could aid in the prevention or treatment of thrombotic events and could also contribute to the development of posthemorrhagic vasoconstriction [6, 11, 18]. It is known that a kind of fatty acid in the lysophospholipid is important factor influencing its biological activity. Therefore, for the synthesis of bioactive LPA with the desired fatty acid, an easy and simple synthetic approach is required. Previously we reported that LPA could be synthesized from glycerol-3-phosphate (G-3-P) with free fatty acid (FFA) by lipase-catalyzed esterification in a solvent free system [7]. LPA synthesis rate and yield in an open reactor system were higher than those in a closed reactor system. Thus, the optimization of LPA production in an open reactor system was performed in our subsequent experiments. Taguchi's methods have found widespread use in industrial process design, principally in development trials, where they are used to generate sufficient process information to establish optimal conditions for a particular process using minimum number of experiment possible. In Taguchi's method, the factors affecting the functional characteristics of the product or process can be divided into two categories: control factors and noise (or uncontrollable) factors. Control factors are the ones which can be easily controlled, e.g. RPM, temperature, molar ratio of substrates, reaction time etc. On the other hand, noise factors are nuisance variables that are either difficult, impossible or expensive to control. Noise factors, in general, are also responsible for causing variance. It is economical to extensively study the environmental variables for the design of a robust process; one that is insensitive to environmental

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disturbances. According to Taguchi's concept, the product must be produced at the optimal levels and with minimal variation in its functional characteristics. Parameter design, a part of Taguchi's method, can be used to make a process robust against sources of variation and hence improve field performance [9, 13].

Controlling noise factors is very costly and cumbersome or impossible. In Taguchi's parameter design, the finding of a prediction equation that is valid over a wide region of the parameter space such as in response surface methodology (RSM) is clearly not the goal. Instead, based on experimental results, the selection of the optimal combination of control factor's level which makes the process less sensitive to the effects of noise factors causing variation of response is the main idea. Because parameter design reduces performance variation by determining the control factor's level that is less sensitive to the sources of variations rather than by controlling the sources of variations themselves, it is a very cost effective technique for improving product quality. The robust design method is used in many areas of engineering. All these showed that this robust design methodology offers simultaneous improvement of process or product quality, performance and cost, and engineering productivity [3, 5, 14, 16]. Its widespread use in industry will have a far-reaching economic impact because this methodology can be applied profitably in all engineering activities including product design and manufacturing process design. Although Taguchi's method is very useful in the optimization of the product design or process design and is used in many other areas, there have been a few cases of application in biotechnology area.

In this paper, we applied Taguchi's method to determine the condition of variables that is less sensitive to changes in environmental conditions and other noise factors for the optimization of lipase-catalyzed production of LPA in an open reactor system.

MATERIALS AND METHODS

Materials

Lipase (Lipozyme IM-46) was kindly donated by Novo Industries (Copenhagen, Denmark). Capric acid (C_{10}) and glycerol-3-phosphate disodium salt (G-3-P) were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). All other reagents were of analytical grade. The following authentic compounds were used as the standards for HPLC analysis, TLC or TLC-FID analysis: lysophosphatidic acid capryl (C_{10}), oleoyl (C_{18}); phosphatidic acid dicapryl (C_{10}), dioleoyl (C_{18}) from Doosan Serdary Research Laboratory (Seoul, Korea).

Methods

Chromatographic assay. We used the assay method developed in our laboratory for monitoring LPA synthesis [8]. Samples were dissolved in chloroform : methanol : water (20 : 10 : 1, by vol) solution, filtered through a PVDF membrane filter (Whatman Co., U.S.A.) and analyzed by HPLC. A Younglin (Seoul, Korea) M 930 pump consisting of a programmable quaternary solvent delivery system was used. An ELSD detector (Alltech, U.S.A.) was attached to the column with the drift tube temperature set at 80°C and gas flow at 2.90 SLPM (standard liter per minute). All analyses were performed using a Hypersil silica column (250 × 4.6 mm, Shandon, England) together with a silica guard column (5 cm × 5 mm).

Taguchi's Parameter Design Method

Objective of Taguchi's method. Taguchi's parameter design can be used to make a process robust against sources of variation and hence improve field performance. If we can design a process that has the robustness to noise factors that largely affects the variance of performance characteristics at a developing stage, it will be very possible for the process to have robustness against other noise factors that could not be considered at the development stage. The aim of a parameter design experiment is, then, to identify settings of the design parameters that maximize the chosen performance measure and are insensitive to noise factors [9].

Orthogonal array. Orthogonal array experiments have the pairwise balancing property where every test setting of a design parameter occurs with every test setting of all other design parameters the same number of times. Orthogonal array experiments minimize the number of test runs while keeping the pairwise balancing property [3]. These basic principles serve as a screening filter which allows the examination of the effects of many process variables, identifying those factors which have a major effects on process characteristics using a single trial with a few reactions. For example, optimization experiment would normally require each variable to be tested independently. Thus, a trial run investigating the effects and interactions of four reaction variables each at three concentration levels, would require an experiment with 81 (i.e. 3^4) separate reactions. Using an orthogonal array, however, an estimate of the effect of each variable can be carried out using only nine experiments. Providing that three level are used for each variable tested, the number of experiments required (E) is calculated from the equation $E=2k+1$, where k is the number of factors to be tested. If the calculated number is not a multiple of three, then the required number of variables to be tested is the next multiple. Hence, as the number of components to be tested is increased, the reduction in the number of experiments required becomes more marked; e.g. to test nine factors would require 3^9=19683 experiments to analyze fully, whereas using Taguchi's methods this could be reduced to just 21 (2×9+1=19, 19 is not a multiple of three and then next integer divisible by three is 21) [4].

Taguchi's parameter design. Taguchi's method for identifying settings of design parameters that maximize performance characteristics (e.g. yield or productivity etc) is summarized as follows; 1) Identify initial and competing settings of design parameters, and identify important noise factors and their ranges. 2) Construct the design and noise matrices, and plan the parameter design experiments. 3) Conduct the parameter design experiments and evaluate the performance statistic for each test run of the design matrix. 4) Use the values of the performance statistic to predict new settings of the design matrix (if needed). 5) Confirm that the new settings indeed improve the performance statistic.

The design will be planned to determine the control factor's level that is less sensitive to noise factors. An orthogonal array containing the control factors will be arranged in the inner array, while an orthogonal array containing noise factors will be arranged in the outer. Taguchi suggested that parameter design using noises that are deliberately created was more effective than not, if noises can be created purposely [13]. The reason is that if noise is not induced deliberately, many experiments must be performed to investigate the effects of noise factors diversely on process and it is very difficult to obtain reliable results under different noise conditions. If the experiments can be performed under various levels of noise, i.e. with positive induction of noise to the design, we can obtain a realistic level of robustness. Therefore, a characteristic of Taguchi's parameter design is the deliberate creation of noise for the identification of control factor's level that is the least sensitive to the noises.

Taguchi's tolerance design. If among the noise factors, some affect the system more profoundly and result in a large variances in performance characteristics, we cannot achieve the combination of control factor's level that is insensitive to all the noise factors. In this case, the noise factor that causes large variance must be controlled to the way of reducing the variance in order to obtain a lower variation, which is called tolerance design according to Taguchi's method. The first step in tolerance design is to determine the contribution of the noise factor to the variation; to identify which noise factors were the cause of large variance. And then, the way of reducing the effect of the noise factor must be considered. Through the tolerance design, we make appropriate economic trade-offs between the increased cost of the product and the improved quality [12].

Taguchi's Parameter Design for Optimization of LPA Production in Open Reactor System

As the control factors that could affect the yield of LPA in an open reactor system, four factors—temperature, molar ratio of substrates, time, and RPM—were selected and as noise factors, three factors—relative humidity, initial water activities of lipase, and G-3-P—were investigated. A complete list of factors and levels is provided in Table 1. The L_9 orthogonal array shown in Table 2 for control factors is the most efficient orthogonal design that accommodates the four factors at three levels. A three level test setting was suitable for revealing nonlinearities in the main effects of the design parameters. The L_9 array specifies that nine experimental runs should be conducted, but the intent is to find the best of the 3^4=81 combinations that exists. This can be done since the design has the orthogonal property that permits the effect of each factor to be separated out. The ones, twos, and threes in the array denote the levels of a

Table 2. L_9 array and assignment of control factors for LPA production in an open reactor system.

	A	B	C	D
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

Table 1. Factors and levels for the parameter design in an open reactor system.

Controllable factors	Levels			Noise factors	Levels	
	1	2	3		1	2
A (temperature, °C)	40	50	60	E (relative humidity)	30%	80%
B (molar ratio of substrate, fatty acid mole/G-3-P mole)	40 : 3	40 : 5	40 : 7	F (a_w of G-3-P)	0.22	0.55
				G (a_w of lipase)	0.22	0.55
C (time, h)	36	48	60			
D (RPM)	200	300	400			

factor. The first test condition in the L_9 array, for example, has all ones across the row, dictating that all the factors should be set at their lowest levels for that particular experiment.

The three noise factors were placed in an L_8 orthogonal array as shown in Table 3. For the columns labeled E, F, and G, the ones and twos in the matrix represent the levels at which those factors should be set during the experiment. The remaining columns are not referenced during the actual experiment, but they can be used during analysis to estimate interaction effects and experimental error. Two arrays were combined as shown in Table 4 to form the complete design layout. The L_9 array containing the control factors is the inner array, while the L_8 array of noise factors is the outer array.

To ensure uniform relative humidity in the chamber, air was circulated with a fan. The relative humidity of chamber was adjusted with saturated aqueous solutions of various salts and monitored by Novasina a_w Center (Novasina, Switzerland). The levels of relative humidity as a noise factor were tested at 30% and 80% ($MgCl_2$ R.H. 30%, $(NH_4)_2SO_4$ R.H. 80%). Also to deliberately induce noise at the initial water level of lipase and substrate, lipase and G-3-P were placed in a closed vessel above a saturated salt solution of known a_w for 7 days at room temperature and the selected a_w levels were 0.22 and 0.55, respectively.

Table 3. L_8 *array and assignment of noise factors and their* interaction for LPA production in an open reactor system.

	E	F	E×F[a]	G	E×G[a]	F×G[a]	e[b]
1	1	1	1	1	1	1	1
2	1	1	1	2	2	2	2
3	1	2	2	1	1	2	2
4	1	2	2	2	2	1	1
5	2	1	2	1	2	1	2
6	2	1	2	2	1	2	1
7	2	2	1	1	2	2	1
8	2	2	1	2	1	1	2

[a] These column are used for calculation of interaction between factors, not for control of factor's level. [b] e is for the estimation of error term.

Lysophospholipid Synthesis Reaction

Under the selected noise conditions, parameter design experiments at various combinations of control factor's levels were performed. Predetermined amounts of the fatty acid were added into the water jacketed reactor which had been thermally equilibrated to a predetermined temperature controlled by water circulation. After melting of the fatty acid, the reaction was started by adding lipase and G-3-P and the reaction mixture was agitated by a mechanical stirrer.

Performance Statistics

In his parameter design, Taguchi uses the signal-to-noise (S/N) ratio as a response [9]. Taguchi stressed the importance of studying the variation of the response by using the S/N ratio in addition to the mean response. In its simplest form, S/N ratio is the ratio of the mean (signal) to the standard deviation (noise) and it also reflects the effect of control factors in relation to the effects of noise factors. The larger the S/N ratio, the better. The S/N ratio simultaneously measures the effect of a factor on mean responses and on variances. In this case, as variance becomes smaller and yield becomes

Table 4. Parameter design for LPA production in an open reactor system.

					Outer Array (L_8)										
				Run	1	2	3	4	5	6	7	8			
				E	1	1	1	1	2	2	2	2			
				F	1	1	2	2	1	1	2	2			
				G	1	2	1	2	1	2	1	2			
Run	Inner Array (L_9)												Responses		
	A	B	C	D									Mean yield[a]	s^2[b]	SN_L[c]
1	1	1	1	1	20.1	12.4	21.5	28.0	24.3	17.0	27.1	12.9	20.4	35.96	25.06
2	1	2	2	2	19.8	23.9	29.2	39.3	19.9	13.9	23.1	14.7	23.0	68.06	25.95
3	1	3	3	3	27.4	31.8	34.4	29.1	18.2	15.0	16.6	13.2	23.2	69.67	25.77
4	2	1	2	3	35.3	32.3	63.9	68.5	53.2	33.4	68.3	65.1	52.5	260.21	33.09
5	2	2	3	1	20.1	26.4	34.5	41.7	40.0	24.5	51.6	33.2	34.0	106.71	29.53
6	2	3	1	2	27.6	25.7	33.4	23.5	30.4	26.3	29.4	23.0	27.4	12.53	28.57
7	3	1	3	2	24.8	25.6	33.1	31.8	54.7	48.6	49.7	56.7	40.6	173.31	30.88
8	3	2	1	3	32.6	23.5	35.5	37.6	47.7	36.5	50.6	41.4	38.2	73.17	30.95
9	3	3	2	1	24.4	30.3	33.6	36.2	30.4	27.2	32.8	26.9	30.2	15.41	29.41

[a] $\dot{y}=\Sigma y_i/n$. [b] $s^2=\Sigma(y_i-\dot{y})^2/(n-1)$. [c] $SN_L=-10 \log_{10}[(1/n)\ \Sigma(1/yi)^2]$.

higher, S/N ratio becomes higher. That is to say, the higher the S/N ratio, the better the process, because it can be a robust process that have a lower variance [9]. The standard formula for this type of response is shown in Eq. (1).

$$S/N_L = -10\log_{10}[(1/n)\sum_{i=1}^{n}(1/y_i)^2] \qquad (1)$$

where y_i refers to the n observations (LPA yields) within an experimental condition of the control factors and n is the number of performance characteristics at a specific control factor level.

The far right side of the column in Table 4 contains nine S/N ratios computed for each of the nine experimental conditions. Since the experimental design is orthogonal (i.e. balanced), it is possible to separate out the main effects in terms of the S/N ratio and in terms of the mean response.

The control factor effects were estimated to determine the relative importance of control factors by computing the respective sum of squares (SS).

$$SS_{A,S/N} = \frac{\sum_{i=1}^{n}(\text{sum of S/N ratios in the i level of A factor})^2}{\text{the number of S/N ratios in the i level of A factor}} - \frac{(\text{total sum of S/N ratios})^2}{\text{the total number of S/N ratios}} \qquad (2)$$

$SS_{B,S/N}$, $SS_{C,S/N}$ and $SS_{D,S/N}$ were calculated as described above.

$$SS_{A,\text{yield}} = \frac{\sum_{i=1}^{n}(\text{sum of yields in the i level of A factor})^2}{\text{the number of yields in the i level of A factor}} - \frac{(\text{total sum of yields})^2}{\text{the total number number of yields}} \qquad (3)$$

RESULTS AND DISCUSSION

Parameter Design for Optimization of LPA Production System

We identified seven factors that could affect the LPA synthesis. Among them, four factors, i.e. temperature, molar ratio of substrates, RPM, and time were chosen as the control factors. Relative humidity and initial water activities of the substrate and lipase could be considered as noise factors in terms of Taguchi's concept. The water level in the reaction system was important in the esterification reaction. Relative humidity represents water vapor pressure of the atmosphere, and may affect the water level of the reaction system. Environmental relative humidity varied with time and hard to control. If the substrates and lipase absorb the water vapor during the storage to a different degree, this may also affect the water level of reaction system and thus cause a variation.

To find out how some factors which can be considered as noise factors influence the system and to find out whether a control factor's level that is less sensitive to the effects of noise factors and gives high yield without control of noise exists, Taguchi's parameter design experiment was performed. We intended to search for the levels of control factors, i.e. temperature, molar ratio of substrates, time, RPM that were least influenced by changes in the condition of noise factors, i.e. relative humidity, initial a_w of G-3-P, and initial a_w of lipase. We decided to test each control factor at three levels and to vary each noise factor over two levels. As described in the Materials and Methods section, two arrays were combined as shown in Table 4 to form a complete parameter design layout. The L_9 array containing the control factors is the inner array, while the L_8 array of noise factors is the outer array. The layout also includes a data matrix, where the yields are recorded. For example, in experimental condition No.1, all the control factors are set at their lowest levels. The first observation yield is 20.1%. This observation was made with E at 30% of relative humidity, F at 0.22 of initial water activity of G-3-P, and G at 0.22 of initial a_w of lipase. The second observation is 12.4% of yield and this observation was made with E at 30% of relative humidity, F at 0.22 of initial a_w of G-3-P, and G at 0.55 of initial a_w of lipase. Hence, the eight observations at each experimental condition of the control factors are not mere repetitions. Each observation was made at a specific combination of noise factors. Therefore, variation due to changes in the noise factors was deliberately induced within each set of eight responses. The far right side column in Table 4 contains nine S/N ratios computed for each of the nine experimental conditions. Since the experimental design is orthogonal (i.e., balanced), it is possible to separate out the effect of each factor. Table 5 shows the result of the analyses of S/N ratios and yield. In terms of S/N ratios, factor A (temperature) is the most significant among control factors. This means that the temperature effect was the largest in these experimental conditions. The average S/N ratios for each level of the four control factors are plotted in Fig. 1A. As for factor A, it does not seem to be much different between A_2 (medium) level and A_3 (high) level. This means that the LPA yield at 50°C was higher than that at 40°C and the LPA yield at 50°C, in turn, was almost the same as that at 60°C. From an economic view point (low temperature is more economical than high temperature), level A_2 (50°C) was chosen as the best option. As indicated above, factors B,

Table 5. Calculation for signal to noise ratios and means in an open reactor system.

		S/N				Yield			
Factor	Level	Sum	Average	SS	% SST[a]	Sum	Mean	SS	% SST[a]
A	A_1	76.78	25.59	47.85	75.0	532.8	22.2	3610.88	52.9
	A_2	91.66	30.33			911.3	38.0		
	A_3	91.24	30.41			872.2	36.4		
B	B_1	89.50	29.83	5.51	8.6	908.3	37.8	1432.06	21.0
	B_2	86.45	28.82			761.2	31.7		
	B_3	83.75	27.92			646.8	27.0		
C	C_1	84.59	28.20	3.20	5.0	688.0	28.7	524.48	7.7
	C_2	88.92	29.64			845.6	35.2		
	C_3	86.19	28.73			782.7	32.6		
D	D_1	84.00	28.00	7.25	11.4	677.1	28.2	1261.75	18.4
	D_2	85.40	28.47			728.1	30.3		
	D_3	90.28	30.09			911.1	38.0		

[a] $SS_T=SS_A+SS_B+SS_C+SS$, % SST for factor A=$(SS_A+SS_T)\times 100$. Percentage of $SS_{T,S/N}$ of control factor B, C, and D were calculated as described above.

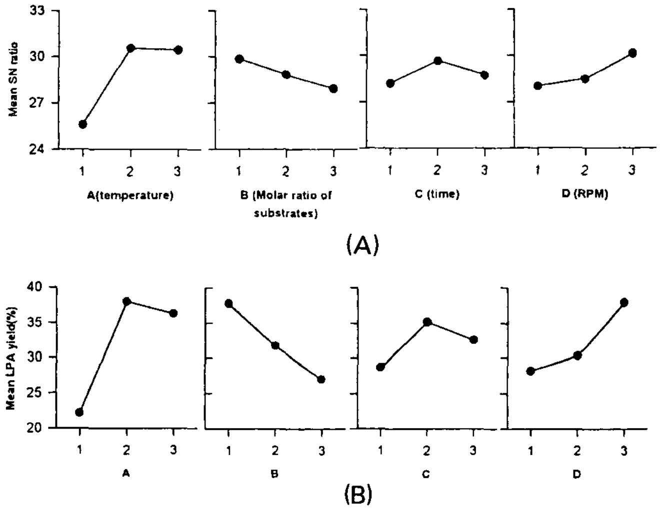

Fig. 1. The effects of control factors on SN_L ratio (A) and mean LPA yields (B).

C, and D appear to be relatively less significant as compared with factor A in terms of the S/N ratio, but level B_1 (fatty acid : G-3-P=50 : 3), C_2 (48 h) and D_3 (400 RPM) are better than any other levels. The purpose of the selection of factor levels is to obtain higher yield and lower variance. The mean response plot of the factors which could aid in the selection of factor levels are shown in Fig. 1B. The mean analysis for factors A, B, C and D suggest that level A_2 (50°C), level B_1 (fatty acid : G-3-P=50 : 3), C_2 (48 h) and D_3 (400 RPM) are better. In terms of B factor (molar ratio of Fatty acid to G-3-P), the smaller G-3-P content, the higher the yield because substrates are readily mixed with less amount of G-3-P and the content of G-3-P which could be converted by lipase is limited. LPA yield did not increase after 48 h. This is because water needed for enzyme reaction was almost evaporated at that time. G-3-P was separated from fatty acid and coagulated after 48 h. The LPA yield was increased at higher RPM. When the mean response, the variation in the response, and economics were simultaneously considered, the levels selected for maximizing LPA yield were A_2 (50°C), B_1 (40 : 3 of molar ratio fatty acid to G-3-P), C_2 (48 h) and D_3 (400 RPM).

Tolerance Design for Optimization for LPA Production System

This combination of control factor's level had to be checked to confirm whether this combination was optimal or not. When the optimal combination is not included in the experimental design condition, the experiment must be newly performed at that combination of control factor levels. The optimal combination determined in our experiment was contained in the experimental design. The results of our analysis are shown in Table 6. The average LPA yield was satisfactory but the variation was too wide to be satisfactory. This means that among the noise factors some caused large variance and there were no combinations of factor levels of temperature, molar ratio of substrates, time and RPM that are insensitive to all noise factors. The noise factor that caused large variance must be controlled in order to obtain a lower variation, which is called tolerance design in Taguchi's method. The first step in tolerance design is to know which noise factors were the cause of large variances. Relative contribution of the noise factor at the optimal factor level condition reveal that F (initial a_w of lipase) factor was the cause of large variance accounting for about 94% of the total variance (Table 7). Thus, a way of reducing the effect of initial a_w of G-3-P must be considered. If the initial water activity of G-3-P is

Table 6. Effect of noise factors at optimal combination of control factor levels.

Run	E (relative humidity)	F (initial a_w of G-3-P)	G (initial a_w of lipase)	LPA yield (%)
1	1	1	1	35.3
2	1	1	2	32.3
3	1	2	1	63.9
4	1	2	2	68.5
5	2	1	1	53.2
6	2	1	2	33.4
7	2	2	1	68.3
8	2	2	1	65.1
				total=420.0 mean=52.5 s^2=266.22

All experiments were performed at the optimal combination of control factor levels [A_2 (50°C), B_1 (molar ratio of substrates (fatty acid mmol : G-3-P mmol=40 : 3), C_2 (48 h), D_3 (400 RPM)].

Table 7. Analysis of variance for noise effect at an optimum condition.

Factor	Sum of square	% SST
E (relative humidity)	50	3.0
F (initial a_w of G-3-P)	1556.8	93.6
G (initial a_w of lipase)	57.2	3.4
Total	1664.0	100

maintained near 0.55, we can greatly reduce the variance of LPA yields. Table 8 shows LPA yields which were obtained by applying tolerance design. By control of the initial a_w of G-3-P to 0.55 at optimal combination of control factors level, we could obtain higher yields (66.5%) and lower variance (5.32) regardless of the levels of other noise factors.

LPA yield at 0.55 of initial a_w of G-3-P was higher than that at 0.22. The reason might be that this reaction system needs some water for reaction but water was evaporated spontaneously in the open reactor system. Thus, relatively higher initial water levels of G-3-P could increase LPA synthesis. LPA synthesis at lower water levels is low because essential water may all be used up at the initial stage of the reaction. Initial a_w of lipase did not affect the LPA yield and variance under this condition. As the reaction progressed, a large amount of lipase got stuck in coagulated G-3-P in the form of a viscous syrup. Thus, initial water of lipase may be taken out by the G-3-P syrup and did not effect LPA synthesis.

Our original goal in this study was to find the control factor's level that gives high yield and low variation without control of all the noise factors considered in our experiment i.e. relative humidity, initial a_w of lipase and initial a_w of G-3-P. If successful, it would be very cost effective. But initial a_w of G-3-P caused a large variance in LPA synthesis in an open reactor system. Thus, among noise factors, at least initial a_w of G-3-P should be controlled so that the process for LPA production gives a lower variance in an open reactor system. In other words, it means that initial a_w of G-3-P which was considered as the one of the noise factors must be considered as a control factor. Through Taguchi's parameter design and tolerance design, we could determine the control factor's level that gives high yield (66.5%) and low variance (5.32). In the mean time, we also studied the optimization of LPA production in a closed reactor system with constant a_w and it will be published soon.

Table 8. LPA yields in case of controlling initial a_w of G-3-P into level 2 (0.55).

Run	E (relative humidity)	F (initial a_w of G-3-P)	G (initial a_w of lipase)	LPA yield (%)
1	1	2	1	63.9
2	1	2	2	68.5
3	2	2	1	68.3
4	2	2	1	65.1
				total=265.8 mean=66.5 s^2=5.32

All experiments were performed at the optimal combination of controllable factor levels [A_2 (50°C), B_1 (molar ratio of substrates (fatty acid mmol : G-3-P mmol=40 : 3), C_2 (48 h), and D_3 (400 RPM)].

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