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Application of Design of Experiments to Homemade Yogurt Production Process

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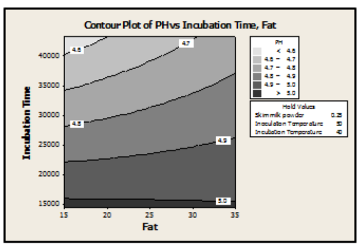
Graphical abstract



Abstract

Design of Experiments (DOE) is a necessary component of product development and improvement, and prepares a scientific approach to evaluation and optimization of experimental factors. This study presents the application of DOE to homemade yogurt production process in order to detect the critical process factors which influence pH values of homemade yogurt and identify the optimal setting for these effective factors. Homemade yogurt were made by changing two different levels of skim milk powder, inoculation temperature, incubation temperature, incubation time, and fat to investigate main and interactions effects on pH of the fermented milk and achieve the optimal pH value from customer prospective in the range of 4.2 to 4.6. The results demonstrated that incubation time and fat are the most effective factors on pH development and the optimal settings for these factors should be 43200 seconds (12 hours) for the incubation time and 17.83 (g/kg) for the fat.

Keywords: Design of experiments; homemade yogurt; pH; response optimizer; case study

Abstrak

Design of Experiments (DOE) adalah komponen penting dalam pembangunan produk dan penambahbaikan serta merupakan pendekatan saintifik untuk penilaian dan pengoptimuman faktor eksperimen. Kajian ini mengolah aplikasi DOE dalam pembuatan pengeluaran yogurt dengan mengesan faktor kritikal yang mempengaruhi nilai pH dan mengenal pasti tahap optimum faktor tersebut. Pembuatan yogurt dibuat dengan mengubah dua tahap berbeza susu serbuk berskim, suhu inokulasi, suhu inkubasi, masa inkubasi, dan lemak dengan menyiasat faktor utama dan kesan interaksi terhadap pH susu fermentasi untuk mencapai pH optimum dari spesifikasi pelanggan pada julat 4.2 sehingga 4.6. Keputusan menunjukkan masa inkubasi dan lemak adalah faktor yang mempunyai kesan utama ke atas pH. Tahap optimum untuk masa inkubasi ialah 43200 saat (12 jam) dan untuk lemak ialah 17.83 (g/kg).

Kata kunci: *Design of experiments;* pembuatan yogurt; pH; pengoptimum respon; kajian kes

1.0 INTRODUCTION

DOE is a systematic powerful technique in order to analyze and determine crucial process factors which are influential in the responses or quality characteristics of the processes and thereby specifying the optimum settings for these factors so as to improve performance of the processes. DOE was created by Sir R.A.Fisher, at the Rothamsted Agricultural Field Research in London, UK in the early of 1920s. At that time, Fisher utilized DOE to determine the best crop by varying and optimizing some parameters such as sunshine, water, amount of fertilizer and soil. After that, this scientific method was developed by means of a lot of researches and studies in various processes and products. In the last 15 years, this method has achieved popularity in the USA and Europe because of improving process capability, decreasing quality costs and enhancing process yield. This method has been widely utilized into various fields of studies. For example, Zalba *et al.* applied DOE to design a thermal energy storage system [14], Chao and Hwang proposed two methods to avoid extra experiments in Tagchui's method and verified that by milling CFRP composite case [15], Gac *et al.* used DOE for the development of an optimum fabrication process for the batch production of thick film titanium oxide- PVC pH electrodes [12]; Lin and Chananda attempt using this method to improve the quality of injection-molded products in manufacturing process [13], and so on. Nowadays, DOE is considered a significant advanced method within the optimization phase of Six Sigma and Design for Six Sigma (DFSS). The main purposes of implementation of DOE in systems and processes include determination of factors which are most impressive on the outputs, determination of influential process factor settings to achieve the best response, and the reduction of variability.

While the application of DOE becomes widespread in many production processes and systems, there are a few studies which have been performed in diverse yogurt production processes by employing DOE to determine key factors influencing improvement of the quality characteristics of various yogurts [1], [2]. Although response surface methodology has been utilized to achieve optimum level of predetermined factors in yogurt studies, diagnosing critical factors which are most effective on quality characteristics of yogurt is still controversial [3], [4], [5], [6]. Accordingly, identification of crucial factors which conclude acceptable and qualified commercial yogurts from customer prospective is significant and also it is regarded as an appropriate prerequisite for response surface methodology. In this study, the application of DOE to homemade yogurt production process was analyzed by utilizing fractional factorial designs which is exerted to specify the most important process factors by executing fewer set of runs than set of runs in full factorial designs. The purpose of application of DOE to homemade yogurt production process in this paper was summarized into two-fold. The first objective was to diagnose and determine the key factors which were most effective on the quality characteristic of homemade yogurt, pH value, and the second objective was to gain the optimal settings of critical factors based on customer perspective.

2.0 EXPERIMENTAL

2.1 Case Study

Nowadays, yogurt represents a significant dairy product all over the world. Yogurt is made by mixing a starter of active yogurt including two types of cultures, Lactobacillus bulgaricus and Streptococcus thermophiles into heated milk. The bacteria convert the milk's sugar, lactose, to lactic acid during fermentation. The lactic acid decreases the pH and makes it slightly sour. Diverse types of yogurt have been produced because of market forces, consumer demands and preference, changing lifestyles, and dietary adjustments. Flavored yogurts were the first and major evolution of yogurt market and inserted a large quantity of types of yogurt to the markets such as fruit flavored [7]. In this study, homemade yogurt which the process of production is somehow similar to manufacturing plain yogurt in factories was utilized.

All quality characteristics in yogurt include texture, color, PH, flavor, viscosity, and composition. Lactic acid produced by fermentation process has an important role in the process of yogurt productions and quality characteristics of final yogurt. Hence, quality control programs in yogurt plants usually include measurements of the rate of the acidity in most of the process. Importance of the acidity increases when acidity of the finished yogurt becomes characterizing criteria for consumers because acidity affects flavors of the yogurt by making it tart. The rate of acidity is measured by titratable acidity tests, but more reliable and prompt tests for acidity measurement are pH measurement by pH Meter [7].

There are some studies regarding quality characteristics of yogurt particularly the rate of acidity and pH level of yogurt. Shaker *et al* studied rheological properties of plain yogurt and effects of fat milk content on increasing the acidity of the plain yogurt. Their experiments resulted that increasing fat milk content reduces rate of decreasing pH during fermentation; Hence, applying high fat milk lead to increasing pH in the finished yogurt [9]. According to Mahdian, E. and Tehrani, M. studies, the growth of starter culture during fermentation has direct relation with acidification. That is, as the bacteria grow, the quantity of acidity in yogurt augments [10]. In another study which was performed by De Brabandere, AG and De Baerdemaeker, JG, the impacts of dry matter fortification such as skim milk powders, heat treatment of the initial milk, starter cultures, and incubation temperatures on changing pH of yogurt during fermentation and finished yogurt were investigated. The results determined that dry matter fortification does not influence pH progression. In addition, incubation temperature and heat treatment affect pH development and treatments of starter culture during incubation time on pH is variable [11].

In the present research, five factors at two levels are selected for application of DOE to homemade yogurt production process. The list of process factors together with their levels which are used for experiments summarized in Table 1. In addition, it was decided to perform experiments in order to determine significant process factors and interactions between them to pH level of homemade yogurt after fermentation as a response. According to studies, consumers prefer to use yogurt with moderate acidity (4.2 to 4.6) [7]. This acceptable range is considered for finished or cooled yogurt; also, cooling yogurt after fermentation of the milk influence to reduce the pH of fermented milk about 2 degrees. Therefore, the range of optimal pH, pH of fermented milk after fermenting and before cooling stage, is designated to be 4.4 to 4.6 as an optimal target for responses in DOE.

Table 1 List of process factors used for the experiment

No	Process Factor	label	Low setting	High setting
1	Skim milk powder (g/1 kg)	A	5	45
2	Inoculation Temperature (°c)	B	50	55
3	Incubation Temperature (°c)	C	40	44
4	Incubation Time (Second)	D	14400	43200
5	Fat (g/1 kg)	E	15	35

2.2 Materials and Methods

2.2.1 Preparation of Homemade Yogurt

Low-fat milk with 15 (g/kg) fat and high-fat milk with 35 (g/kg) fat were prepared from Dutch Lady Plant and were homogenized. The samples were provided by blending appropriate amounts of milk with 5 (g/kg) and 45 (g/kg) skim milk powder. The mixtures were heated at 85°C for 600 seconds (10 min). Starter culture was provided from commercial, unflavored yogurt, Nestle plant. For making homemade yogurt, plain yogurt can be used for starter culture [8]. Milk samples were cooled at two levels of inoculation temperatures, 50°C and 55°C, by storing in the refrigerator. Samples of 950 ml of specified milk compositions were inoculated with 50 ml of the starter culture at aforementioned inoculation temperatures. The inoculated milk samples were incubated at 40°C and 44°C until 14400 second (4 hours) and 43200 seconds (12 hours) by microwave.

2.2.2 PH Measurement

The pH values of the yogurt samples at the end of incubation time was measured by dipping the glass electrode of the pH meter (Sartorious AG, PB-10) into the milk. The pH meter was cleaned between measurements by water and was calibrated with buffers before measuring the next sample. All experiments were made with 450 ml of yogurt in a glass beaker.

2.2.3 Experimental Design and Statistical Analysis

A $2^{(5-1)}$ fractional factorial design with resolution V at two replicates was used to investigate main and interactions effects on PH of the yogurt. The experiments were performed in two replicates, totally 32 runs. Furthermore, each replicate were carried out in one block, that is the first replicate were run on one day and second replicate on another day. The Minitab, version 15, was used to present all statistical analysis.

3.0 RESULTS AND DISCUSSION

3.1 Design of pH Experiment and Identification of Significant Process Factors

The design and the results of experiments, which are the pH values of fermented milk as a response, are shown in Table 2. The design generator is E = ±ABCD and the defining relation is I = ABCDE. In this fractional factorial design, the effects and interaction effects which are confounded with each other, that are called aliases, are given in Table 3. The analysis of variance for this experiment which is obtained from Minitab is presented in Table 4. It was decided to choose the significance level (α) 5 per cent to determine significant factors and interactions. In Analysis of Variance table, if the p-value becomes less than the significance level (α), the factor or the interaction effect is then regarded to be statistically significant, on the other hand, if the p-value becomes greater than α, it is concluded that the factor or interaction effect are not significant. For the pH experiment, main effects D and E and the interaction effect D×E are significant because the p-values are less than 0.05 (as shown in right highlighted figures in Table 4). From evaluating the effects of significant factors and interactions in Table 4 (as shown in left highlighted figures), it is indicated that incubation time (D) has highest effect on pH value in proportion to other effects. In addition, changing incubation time from low to high level reduces pH value because of negative effect. Fat (E) has second highest effect on pH value; also changing fat level from low to high level increases pH value due to positive effect. Finally, the effect of D×E interaction is smallest and has p-value of 0.043. It means that this interaction is significant at the 0.05 α-level.

3.2 Analysis of Main Effects and Interactions Trends

From comparison among main effects plot which is shown in Figure 1, it is inferred that when fat raise from low level to high level, the pH of yogurt increases. In addition, shifting from low level of incubation time to high level causes pH of yogurt to be decreased. Some studies confirm the treatments of incubation time and fat versus pH. According to Shaker *et al*. studies, when the milk fat content increases the rate of decreasing pH during fermentation of yogurt diminishes [9]. This finding corresponds to the trend of fat level in the main effect plot. That is, when fat raise from low level to high level, the pH of yogurt increases. In addition, Figure 1 shows that shifting from low level of incubation time to high level causes pH of yogurt to be decreased. Reduction of pH value followed by increase of incubation time has been demonstrated by previous studies [7]. It must be pointed out that in addition to confirmation previous studies about treatment of incubation time and fat versus pH value, these experiments illustrate that these two factors are most effective factors on pH values of the yogurt in proportion to other factors.

Table 2 $2^{(5-1)}$ design for the PH experiments

Run	A	B	C	D	E=AB CD	Replicate 1	Replicate 2
1	5	50	40	14400	35	5.04	4.98
2	45	50	40	14400	15	4.96	5.01
3	5	55	40	14400	15	4.92	4.87
4	45	55	40	14400	35	5.35	4.91
5	5	50	44	14400	15	4.89	5.07
6	45	50	44	14400	35	4.95	4.89
7	5	55	44	14400	35	4.98	4.92
8	45	55	44	14400	15	5.01	5.07
9	5	50	40	43200	15	4.41	4.69
10	45	50	40	43200	35	4.71	4.85
11	5	55	40	43200	35	4.66	4.81
12	45	55	40	43200	15	4.51	4.65
13	5	50	44	43200	35	4.47	4.85
14	45	50	44	43200	15	4.49	4.55
15	5	55	44	43200	15	4.53	4.44
16	45	55	44	43200	35	4.96	4.85

Table 3 Alias structure for the $2^{(5-1)}$ design with resolution V

Process Factor	Label	Low-level Setting
A = BCDE	AB = CDE	BD = ACE
B = ACDE	AC = BDE	BE = ACD
C = ABDE	AD = BCE	CD = ABE
D = ABCE	AE = BCD	CE = ABD
E = ABCD	BC = ADE	DE = ABC

Table 4 Analysis of variance output from Minitab for pH experiments

Fractional Factorial Design

Estimated Effects and Coefficients for Response(PH) (coded units)

Term	Effect	Coef	SE Coef	T	P
Constant		4.8203	0.02357	204.50	0.000
Block		-0.0178	0.02357	-0.76	0.462
A	0.0744	0.0372	0.02357	1.58	0.136
B	0.0394	0.0197	0.02357	0.84	0.417
C	-0.0256	-0.0128	0.02357	-0.54	0.595
D	-0.3369	-0.1684	0.02357	-7.15	0.000
E	0.1319	0.0659	0.02357	2.80	0.014
A*B	0.0731	0.0366	0.02357	1.55	0.142
A*C	0.0031	0.0016	0.02357	0.07	0.948
A*D	0.0144	0.0072	0.02357	0.30	0.765
A*E	0.0206	0.0103	0.02357	0.44	0.668
B*C	0.0356	0.0178	0.02357	0.76	0.462
B*D	0.0094	0.0047	0.02357	0.20	0.845
B*E	0.0481	0.0241	0.02357	1.02	0.324
C*D	0.0069	0.0034	0.02357	0.15	0.886
C*E	-0.0294	-0.0147	0.02357	-0.62	0.543
D*E	0.1044	0.0522	0.02357	2.21	0.043

Analysis of Variance for Response(PH) (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Blocks	1	0.01015	0.01015	0.01015	0.57	0.462
Main Effects	5	1.10892	1.10892	0.22178	12.47	0.000
2-Way Interactions	10	0.17173	0.17173	0.01717	0.97	0.508
Residual Error	15	0.26670	0.26670	0.01778		
Total	31	1.55750				

The strong interaction between incubation time (D) and fat (E) is shown in Figure 2. This plot indicates that, see lower interaction plot in Figure 2, the decrease in pH by moving from the low to the high level of incubation time is greater when the fat level is low (solid line) than when it is high (dash line). In other words, the slope of reducing pH is further at low level of fat while moving from low level of incubation time to high level. This finding clearly approved the results of Shaker *et al*. studies about relation between fat level and acidity of yogurt. 3-ways and 4 ways interaction are considered negligible in this experiment.

3.3 Calculation of Optimal Process Factor Settings

The determination of optimal process factor settings which influence pH value, in order to be located in the range of 4.4 to 4.6, was decided to conduct by Minitab Response Optimizer. The objective for pH values is to achieve a quantity at or next to the target value of 4.5.

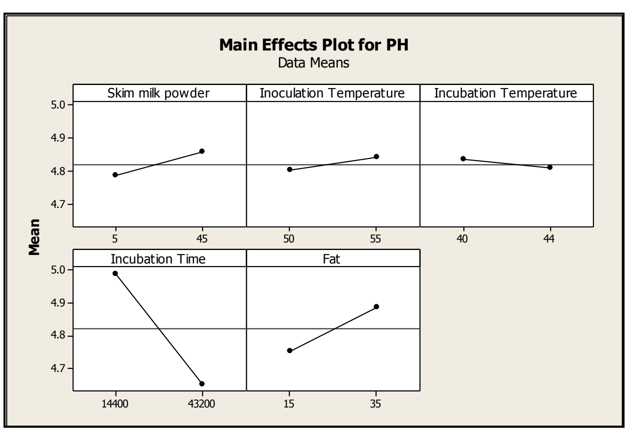


Figure 1 Main effects plot

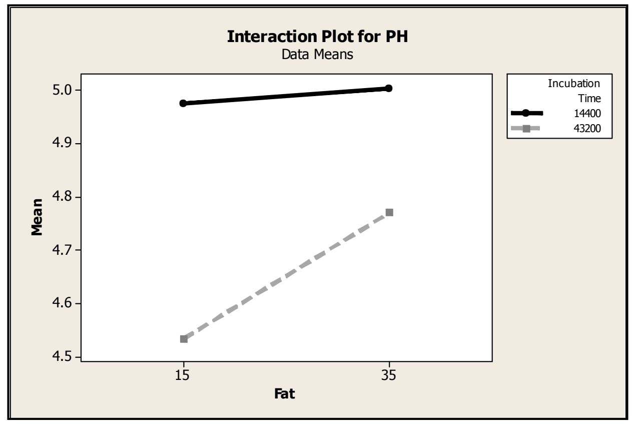


Figure 2 Interaction plot

Therefore, the pH values in the range of the specification limits, 4.4 to 4.6, are satisfactory and the pH values less than 4.4 or higher than 4.6 are not acceptable. By using response optimizer of Minitab, it is concluded that the optimal process factor settings are achieved in high level of incubation time (D), 43200 seconds (12 hours), and relatively low level of fat percentage (E), 17.83 (g/kg). Furthermore, results in Table 5 shows that the desirability of this optimal arrangement is equal to one; it indicates that the predicted response, y, by the value of 4.5, is completely close to the target requirements. After specification of level of process factors for reaching to optimal target of pH value, it was decided to execute five more experiments for confirmation of findings. The methodology which applied for performing more experiments was completely corresponding to aforesaid methods in section 2; however, the factors of incubation time (D) and fat (E) were selected in high level, 12 hours, and relatively low level, 17.83 (g/kg), correspondingly. The pH values of fermented milk were 4.53, 4.51, 4.49, 4.51, and 4.53. These results indicate that the significant controllable factors, which are most effective on pH values, are incubation time and fat. Moreover, for reaching to the optimal range and target of the pH values, the high level of incubation time and the relatively low level of fat should be considered.

Table 5 Response Optimization output from minitab for PH experiments

Response Optimization

Parameters

	Goal	Lower	Target	Upper	Weight	Import
PH	Target	4.4	4.5	4.6	1	1

Global Solution

Skim milk Powder = 5
Inoculation Temperature = 55
Incubation Time = 43200
Incubation Temperature = 41.9025
Fat = 17.8368

Predicted Responses
PH = 4.5 , desirability = 1.000000
Composite Desirability = 1.000000

3.4 Model Development

Reduced regression model based on significant factors and interactions, incubation time (D), fat (E) and D×E interaction is Equation (1)

$$Y = \beta_0 + \beta_4 X_4 + \beta_5 X_5 + \beta_{45} X_4 X_5 \quad (1)$$

Where y is response (pH), X_4 is incubation temperature (D), X_5 is fat (E), and X_4X_5 is D×E interaction. In addition, the coefficients β_0, β_4, β_5, and β_{45} are identified the regression coefficients. The regression coefficients β_4, β_5, and β_{45} are estimated from one-half of the corresponding effects and β_0 from the grand average of all 32 observations. The estimation of response or predicted value for pH is Equation (2)

$$\hat{Y} = 4.8203 - 0.1684\ X_4 + 0.0659\ X_5 + 0.0522\ X_4X_5 = 4.8203 - 0.1684\ (+1) + 0.0659\ (-1) + 0.0522\ (+1)\ (-1) = 4.5338 \quad (2)$$

It is clear that this value of pH is in the range of desirable pH value, 4.4 to 4.6, and is close to the optimal target of pH, 4.5.

Surface Plot or the plane which illustrates the predicted pH values based on regression model is shown in Figure 3. This figure indicates that for achieving to optimal range of pH, 4.4 to 4.6, the quantities near the high level of incubation time and low level of fat should be selected. However, in the previous section, the optimal factor settings are resulted in the relatively high level of incubation time and the low level of the fat. Contour Plot is shown in Figure 4. The lightest gray area in the Contour Plot indicates the regions in which predicted pH values produced by regression model are in the optimal range. This region contains points that have the PH values of 4.4 to 4.6. However, by referring to previous section, the optimal target pH value, 4.5, gained in high level of incubation time and low level of fat.

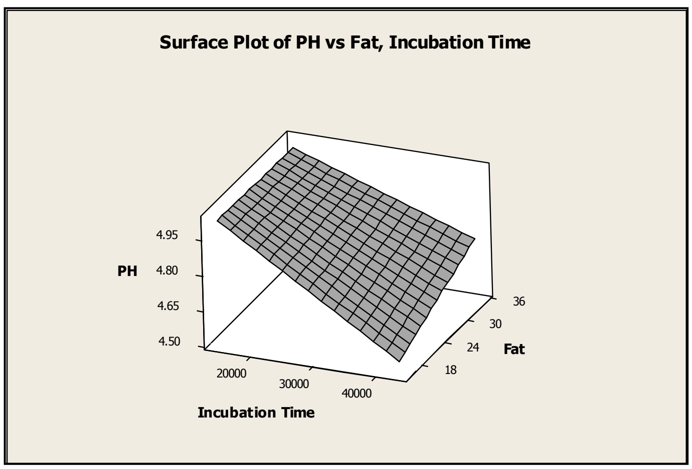


Figure 3 Surface plot

4.0 CONCLUSION

Two objectives of this study were determination of significant process factors in homemade yogurt production process and specification of optimal settings for these factors. The crucial factors which are most effective on pH values of homemade yogurt were incubation time and fat. Moreover, in order to achieve the optimal range of pH value in homemade yogurt, 4.4 to 4.6, the optimal settings for these factors should be 43200 seconds (12 hours) for the incubation time and 17.83 (g/kg) for the fat. The results of the study can direct researchers to apply response surface methodology and other optimization techniques for future researches.

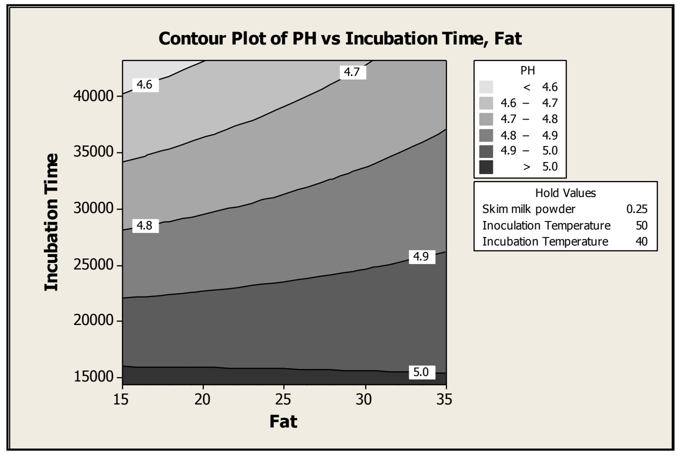


Figure 4 Contour plot

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