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Optimization of L-methionine Bioconversion to Aroma-active Methionol by *Kluyveromyces lactis* Using the Taguchi Method

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Abstract

The bioproduction of methionol through fermentation was performed by *Kluyveromyces lactis* KL71 in coconut cream supplemented with L-methionine (Met). This bioprocess was successfully optimized with the Taguchi method applying the L_{27} (3^{13}) orthogonal array. Among these studied factors, shaking speed was found to be the most significant factor that affected the bioproduction of methionol, followed by incubation time, pH level and Met concentration. The optimum fermentation conditions were determined as follows: 0.45% (w/v) of Met, 48 h of incubation, shaking speed of 160 rpm, 0.05% (w/v) of yeast extract (YE), 0 mg/L of diammonium phosphate (DAP) and pH of 6.3. Under the optimum conditions, the signal to noise (S/N) ratio achieved was 59.9 decibels (dB), which was in good agreement with the predicted S/N ratio of 58.2 dB. The average yield of methionol obtained under the optimum fermentation conditions was 990.1 ± 49.7 μg/mL. This indicates that the Taguchi method was effective for the optimization of bioproduction of methionol by *K. lactis*.

Keywords: methionol, L-methionine, bioproduction, *Kluyveromyces lactis*, Taguchi method

1. Introduction

Volatile sulphur flavour compounds (VSFCs) are important sulphur-containing aroma compounds that play a considerable role in the flavour of many food products (Ugliano & Henschke, 2009; Yvon & Rijnen, 2001). Some of the VSFCs commonly found in food products are methanethiol (MTL), dimethyl disulphide (DMDS), dimethyl trisulfide (DMTS), 3-methylthio-1-propanal (methional) and 3-methylthio-1-propanol (methionol). The flavour compound of interest in this study was methionol, which has a distinct cauliflower- and cabbage-like aroma when present at levels exceeding its threshold level of 1 ppm in wine (Mestres, Busto, & Guasch, 2000). Although the presence of methionol may be detrimental to the quality of wines, this compound is in fact of great importance in the overall aroma of soy sauce and cheese (Ugliano et al., 2009; Yvon et al., 2001).

The production of flavour compounds via chemical synthesis is commonly practiced but its major disadvantage is the production of undesirable racemic mixtures (Vandamme & Soetaert, 2002). In addition, health-conscious consumers may have an aversion to the use of chemically synthesized flavourings in food products. Hence, the flavour industry has realized the importance of moving towards developing flavours through bioprocesses such as fermentation, where microorganisms are exploited for the bioconversion of precursors such as amino acids to form desirable flavour compounds. Methionol can be produced by the bioconversion of sulphur-containing amino acids like L-methionine (Met) via Ehrlich pathway which is shown in Figure 1 (Landaud, Helinck, & Bonnarme, 2008).

The Ehrlich pathway is an enzymatic pathway that results in the formation of methionol. This pathway starts with Met undergoing a transamination process to form α-keto-γ-(methylthio) butyric acid (α-KMBA), followed by a decarboxylation process to form methional, which is then subsequently reduced to form methionol (Landaud et al., 2008; Lopez del Castillo-Lozano, Delile, Spinnler, Bonnarme, & Landaud, 2007). Besides methionol, minor VSFCs such as MTL, DMDS, DMTS, dimethyl tetrasulphide (DMQS),

3-methylthio-1-propionic acid (3-MTP acid) and 3-methylthio-1-propionic acetate (3-MTPA) may also be produced (Figure 1).

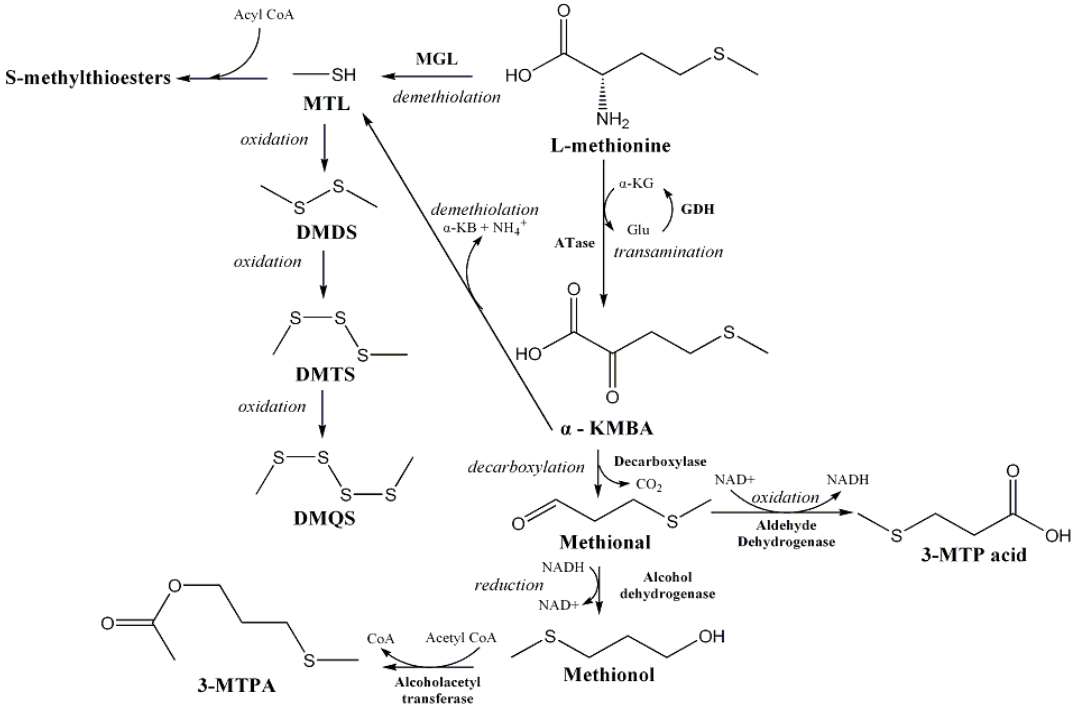

Figure 1. Metabolic pathways of Met catabolism to produce VSFCs. ATase, aminotransferase; α-KB, alpha-ketobutyrate; α-KG, alpha-ketoglutarate; Glu, glutamate; GDH, glutamate dehydrogenase; α-KMBA, α-keto-γ-(methylthio) butyrate; MGL, methionine γ-lyase; MTL, methanethiol; DMDS, dimethyl disulphide; DMTS, dimethyl trisulphide; DMQS, dimethyl tetrasulphide; 3-MTP acid, 3-methylthio-1-propionic acid; 3-MTPA, 3-methylthio-1-propyl acetate (Etschmann, Kötter, Hauf, Bluemke, Entian, & Schrader, 2008; Landaud, et al., 2008; Lopez del Castillo-Lozano et al., 2007)

In this study, *Kluyveroymces lactis* (*K. lactis*) was employed for the production of methionol because it is well known to contribute to the production of VSFCs in the cheese-ripening process (Landaud et al., 2008). The bioproduction of methionol from *K. lactis* yeast was previously optimized using the classical method of varying one variable at a time while keeping the others at a predetermined level (Seow, Ong, & Liu, 2010). However, this traditional method is inefficient at times as it requires numerous experiments that are both labour- and time-intensive. Therefore, multivariate techniques such as response surface methodology (RSM) and the Taguchi method are commonly utilized as tools for optimization processes. The working principle of RSM requires the pre-screening of variables to identify those that have significant effects on the outcome (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008). Although RSM utilizes a multi-factorial experimental design for optimization, it is a challenge to study many variables at one time by RSM as this methodology would require a large number of experiments.

The application of fractional factorial design methods such as the Taguchi method allows a more flexible way of studying several parameters using a small number of experimental trials (Ross, 1996). This is achieved by the design of an orthogonal array (OA) that is reported to be both efficient and cost-effective (Peace, 1993). Although the Taguchi method has been widely applied in the manufacturing industry, this method has only been explored, to a limited extent, as a way of optimizing processes in fermentation and food processing (Prakasham, Rao, Rao, & Sarma, 2005; Rao, Prakasham, Prasad, Rajesham, Sarma, & Rao, 2004). A recent review also highlighted the application of the Taguchi method in biotechnology where the method was shown to be successful in increasing the efficiency of several biotechnological processes (Rao, Kumar, Prakasham, & Hobbs, 2008).

Due to the aforementioned advantages of the Taguchi method, this method was exploited in the present work to optimize methionol bioproduction by *K. lactis*. Although the Taguchi method using orthogonal array (OA) to

design experiment allows the study of interactive effects between factors, the working power of an OA design is limited compared to a full factorial design. Nevertheless, leveraging off the attractive potential of the Taguchi method in fermentation processes, it was interesting to explore the application of this method in the current study. The objective of the present study was to optimize the fermentation conditions using the Taguchi method to produce substantial amounts of methionol via L-methionine catabolism by *K. lactis* yeast in coconut cream using an OA layout of L_{27} (3^{13}). Coconut cream was chosen as the fermentation medium as it contains sufficient nutrients for yeast growth and is high in fat which retains volatile flavour compounds.

2. Method

2.1 Media and Preculture Preparation

The nutrient broth consisted of 0.25% (w/v) of Bacto™ yeast extract (YE) (BD, Franklin Lakes, NJ, USA), 0.25% (w/v) of malt extract (Oxoid, Basingstoke, UK), 0.25% (w/v) of peptone (Oxoid, Basingstoke, UK) and 2.0% (w/v) of glucose (Glucolin®, Glaxo Wellcome Celon Limited, Moratuwa, Sri Lanka) was used to prepare the pure culture. The pH level of the nutrient broth was adjusted to 5.0 using 1.0 M hydrochloric acid (HCl). Two loopfuls of freeze-dried *K. lactis* KL71 yeast (Danisco, Singapore) were added into the 15 mL portion of sterilized nutrient broth (121 °C for 15 min) and incubated at 30 °C for 24 h. The pure culture was then dispensed in 1.5 mL aliquots into sterilized culture vials that were kept at -80 °C before use.

The frozen pure yeast culture was thawed and 1.0 mL of it was added into 100-mL bottles containing 20 mL of UHT-processed and aseptically packed coconut cream which functioned as the experimental preculture medium (25.4% fat, 4.6% protein, 1.3% carbohydrate, Kara, Fairteck Holding Pte. Ltd., Singapore). After incubation for 24 h at 30°C, spread plating on potato dextrose agar (PDA) plates was conducted to determine cell counts in the culture. The preculture with a cell count of about 10^7 CFU/mL was obtained and used for the fermentations.

2.2 Fermentation Conditions and Experimental Design

Table 1. Parameters and their levels employed in the Taguchi method for optimizing the bioproduction of methionol

Parameters	Levels		
	1	**2**	**3**
A: Met (% w/v)	0.15	0.30	0.45
B: Incubation time (h)	24	48	72
C: Shaking speed (rpm)	0	80	160
D: YE (% w/v)	0	0.05	0.20
E: DAP (mg/L)	0	250	500
F: pH	4.0	5.0	6.3

Fermentation was carried out by inoculating 1% (v/v) of preculture into the experimental culture media which was 40 mL of coconut cream. The experimental culture media was supplemented with different concentrations of Met (≥ 99%, non-animal source, Sigma, Unterhaching, Germany), YE and diammonium phosphate (DAP) (≥ 98%, Sigma, Japan) and was subjected to different treatments according to the experimental design. A total of six parameters were studied including Met concentration (% w/v), incubation time (h), shaking speed (rpm), YE concentration (% w/v), nitrogen level by addition of DAP (mg/L) and pH level. In addition to the main effects of the parameters studied, two interactive effects between parameters were also investigated: Met level and incubation time (A × B), and incubation time and shaking speed (B × C). The studied parameters and their levels are shown in Table 1. An OA experimental design of L_{27} (3^{13}) was employed. The experimental design and data analysis (Table 2) was performed using Minitab software (Version 16, Minitab inc., PA, USA). All fermentations were carried out in triplicate in a fermentation volume of 50 mL.

Table 2. Experimental results of the methionol yields and calculated signal to noise (S/N) ratios (Taguchi OA of L-27)

Run	A: Met	B: Incubation time	C: Shaking speed	D: YE	E: DAP	F: pH	Average yield (μg/mL)	S/N ratio (decibel, dB)
1	1	1	1	1	1	1	34.1	29.3
2	1	1	2	2	2	2	72.5	37.0
3	1	1	3	3	3	3	447.7	52.8
4	1	2	1	1	1	2	119.2	41.1
5	1	2	2	2	2	3	113.7	40.5
6	1	2	3	3	3	1	346.9	49.7
7	1	3	1	1	1	3	188.0	45.0
8	1	3	2	2	2	1	162.0	42.2
9	1	3	3	3	3	2	414.1	52.2
10	2	1	1	2	3	2	80.1	38.0
11	2	1	2	3	1	3	105.4	40.1
12	2	1	3	1	2	1	329.3	49.7
13	2	2	1	2	3	3	167.1	43.6
14	2	2	2	3	1	1	127.3	40.8
15	2	2	3	1	2	2	670.0	56.1
16	2	3	1	2	3	1	155.0	41.9
17	2	3	2	3	1	2	269.4	45.5
18	2	3	3	1	2	3	655.7	55.9
19	3	1	1	3	2	3	110.4	40.8
20	3	1	2	1	3	1	44.3	32.4
21	3	1	3	2	1	2	678.4	56.0
22	3	2	1	3	2	1	97.7	39.8
23	3	2	2	1	3	2	170.4	44.4
24	3	2	3	2	1	3	859.4	58.2
25	3	3	1	3	2	2	190.4	44.5
26	3	3	2	1	3	3	287.8	49.1
27	3	3	3	2	1	1	559.6	54.7

To investigate the effect of Met concentration on the bioproduction of methionol, 0.03 g/mL of Met water solution was added to 40 mL of the coconut cream culture media in 2.5 mL, 5.0 mL and 7.5 mL portions to achieve the final concentrations of 0.15%, 0.30% and 0.45% (w/v), respectively. The effect of YE level was studied by adding 0.5 mL of 0.05 g/mL and 1.0 mL of 0.10 g/mL of YE water solution to the coconut cream to attain the final concentrations of 0.05% and 0.20% (w/v), respectively. In addition, the effect of nitrogen supplementation on methionol production was examined by adding 0.5 mL and 1.0 mL of DAP water solution at a concentration of 25.0 mg/mL in order to achieve the final concentrations of 250 mg/L and 500 mg/L, respectively. This would in turn produce 53.0 mg/L and 106.1 mg/L of yeast assimilable nitrogen (YAN). In experimental trials where YE or DAP was at its lowest level, it was replaced by adding sterile deionized water instead (i.e. no supplementation). The final fermentation medium volumes were fitted to 50 mL with sterilized deionized water. Aeration effect was also studied by subjecting cultures to 3 different shaking speeds (0, 80 and 160 rpm) in the shaking water bath (SW22, Julabo Labortechnik GmbH, Seelbach, Germany). To terminate the

fermentation process, 1.0 M HCl was added to decrease the pH value to 2.5. The samples were then immediately stored at -20 °C until analysed.

2.3 Sample Analysis

Headspace solid-phase microextraction (HS-SPME) was used to extract the volatile flavour compounds from fermented samples. Five millilitres of sample diluted with deionized water (10-20 times) was put into a 20-mL SPME vial for HS extraction and analysis. The adsorption of volatile compounds was carried out with an 85 μm carboxen-polydimethylsiloxane (CAR-PDMS) fibre (Supelco, Bellefonte, PA, USA). The SPME fibre was incubated with the samples at 80 °C for 30 min (shaking speed 250 rpm) to adsorb volatile compounds. After adsorption, the fibre was desorbed at the injector port and the released volatile compounds were analyzed using an Agilent (Palo Alto, CA, USA) 6890N network gas chromatography (GC) system equipped with a DB-FFAP capillary column (60 m × 0.25 mm × 0.25 μm), 5975 inert mass selective detector (MSD) and flame ionization detector (FID). The carrier gas used was purified helium at a flow rate of 1.2 mL/min. The oven temperature was set at 50 °C for 5 min, after which it was ramped to 230 °C at 5 °C/min and kept for 10 min. Splitless mode was applied, and the injector and detector temperatures were set at 250 °C. The mass spectra information obtained from MSD was used for the identification of volatile compounds. The concentrations of methionol in all fermented samples were determined based on the peak area determined by FID. The calibration curve was established using external standard method by plotting FID peak areas against methionol (≥ 98%, Sigma-Aldrich, St. Louis, MO, USA) standard concentrations.

2.4 Taguchi Method and Analysis of Variance (ANOVA)

The methionol concentrations in fermented coconut cream were determined and the corresponding S/N ratios were calculated based on the larger-the-better S/N ratio calculation formula as defined (Equation 1) (Adnani, Basri, Malek, Salleh, Abdul Rahman, Chaibakhsh, & Rahman, 2010):

$$\text{S/N ratio} = -10\log\left(\frac{1}{r}\sum_{i=1}^{r}\frac{1}{y_i^2}\right) \quad (1)$$

where r represents the total number of tests in the orthogonal array, and y_i is the observed data.

ANOVA was performed to determine the parameters that were statistically significant in affecting the bioproduction of methionol. In addition, the main effect and interaction plots were constructed to determine the optimum fermentation conditions. Under the determined optimum conditions, the predicted S/N ratios were calculated based on the Taguchi prediction formula (Peace, 1993). Confirmation runs were carried out in triplicate under the optimum fermentation conditions to validate the experimental design so that if the results of the confirmation runs were close to the predicted values, the optimum fermentation conditions can be implemented (Adnani et al., 2010). A large-volume fermentation of 500 mL was also carried out under the optimum fermentation conditions.

3. Results and Discussion

3.1 Experimental Results

Table 3. Mean S/N ratios at different levels for each parameter

Parameters	Mean S/N ratio (dB)				Rank
	Level 1	Level 2	Level 3	δ	
A: Met	43.3	45.7	46.7	3.4	4
B: Incubation time	41.8	46.0	47.9	6.1	2
C: Shaking speed	40.4	41.4	53.9	13.5	1
D: YE	44.8	45.8	45.1	1.0	5
E: DAP	45.6	45.2	44.9	0.7	6
F: pH	42.3	46.1	47.3	5.0	3

The experimental data and the corresponding S/N ratios were shown in Table 2. In an orthogonal experimental design, the effect of each parameter at the respective levels could be separated out (Zhou, Wu, & Guo, 2010). For instance, the mean S/N ratios for Met supplementation at levels 1, 2 and 3 were determined by calculating the average S/N ratios for experimental runs 1 to 9, 10 to 18, and 19 to 27, respectively. Similarly, the mean S/N ratios for the remaining parameters at their respective levels were also computed. As such, the S/N ratio responses are shown in Table 3. The mean S/N ratio range for each parameter was calculated by taking the difference in S/N ratios between the levels that obtained the highest mean S/N response and the lowest mean S/N response. This range is represented by δ value. Determination of these δ values is important for ranking the parameters from the strongest to the weakest effect on the bioproduction of methionol where a high δ value would indicate that the parameter had a strong impact on the output signal response (Peace, 1993). From the experimental results, it was observed that shaking speed had the strongest effect on the bioproduction of methionol since its δ value was the highest. However, the effects of parameters like YE and DAP supplementation were weaker as their δ values were lower compared to the rest.

Table 4. ANOVA results based on the obtained experimental data (S/N ratios)

Parameters	**[a]DF**	**Sum of squares**	**Mean-square**	**F-value**	**[b]Prob>F *p*-value**
A: Met	2	54.28	27.14	6.44	0.032
B: Incubation time	2	176.76	88.38	20.96	0.002
C: Shaking speed	2	1021.18	510.59	121.08	<0.001
D: YE	2	4.72	2.36	0.56	0.599
E: DAP	2	2.38	1.18	0.28	0.764
F: pH	2	124.71	62.36	14.79	0.005
A×B	4	2.78	0.70	0.17	0.949
B×C	4	50.62	12.65	3.00	0.111
Residual Error	6	25.30	4.22		
Total	26	1462.73			

[a] Degree of Freedom.

[b] ANOVA was conducted at confidence level of 95% with α value of 0.05.

In addition, statistically significant factors were determined through ANOVA. As listed in Table 4, the model terms that were statistically significant in this study were Met concentration, pH, incubation time and shaking speed, which were ranked with increasing significance. Interestingly, the ranking sequence of significant parameters obtained from ANOVA was consistent with the ranking of influential parameters in Table 3. It was also observed that the studied interactions were not statistically significant, implying that the interactions between the assigned parameters were not distinct enough to affect the bioproduction of methionol.

3.2 Main Effects and Interaction Plots

The main effect plots corresponding to the different parameters are presented in Figure 2. The plots display the corresponding mean S/N ratios of the respective levels of the parameters. Since Met is the precursor of methionol in Ehrlich pathway, a higher Met concentration would lead to the production of higher amounts of methionol. Indeed, as shown in Figure 2, such a trend was observed and was in agreement with several other studies (Liu & Crow, 2010; Moreira, Mendes, Pereira, Guedes de Pinho, Hogg, & Vasconcelos, 2002; Quek, Seow, Ong, & Liu, 2011; Seow et al., 2010).

Incubation time is closely related to the growth and metabolism of the yeast. From Figure 2, it can be seen that longer incubation times resulted in higher methionol production. In an investigation conducted by Jiang on the fermentation by *K. lactis*, the changes of volatiles in the fermentation medium were reported with increasing incubation time (Jiang, 1995). These volatiles produced during fermentation are normally secondary metabolites which are usually biosynthesized when the growth of the yeast is limited due to nutrient exhaustion (Robinson, Singh, & Nigam, 2001). However, Jiang's study reported that the production of volatile secondary metabolites

by *K. lactis* was associated with its growing biomass (Jiang, 1995). Therefore, this suggests that the production of volatile secondary metabolites such as methionol may start during the initial growth phase of *K. lactis* but not necessarily start if and when the growth of *K. lactis* is limited. Similarly as observed in this study, the increasing incubation time resulted in the bioproduction of higher amounts of methionol. In this study, a correlation between methionol production and biomass could not be deduced as the cell count measurement of the experimental culture was not determined throughout the fermentation process. Therefore, a further study is required to investigate the relationship between methionol biosynthesis and yeast biomass.

A sharp increase in S/N ratio was observed as the shaking speed increased from 80 to 160 rpm, with a maximum methionol concentration obtained at 160 rpm (Figure 2). This is likely due to the aerobic nature of the *K. lactis* yeast (a respirofermentative yeast), where substantial shaking would provide sufficient oxygen for their growth and metabolism. This phenomenon was consistent with that observed in another study where the amount of methionol produced in the aerated ferment was nearly 5 times more than that in non-aerated ferment (Seow et al., 2010).

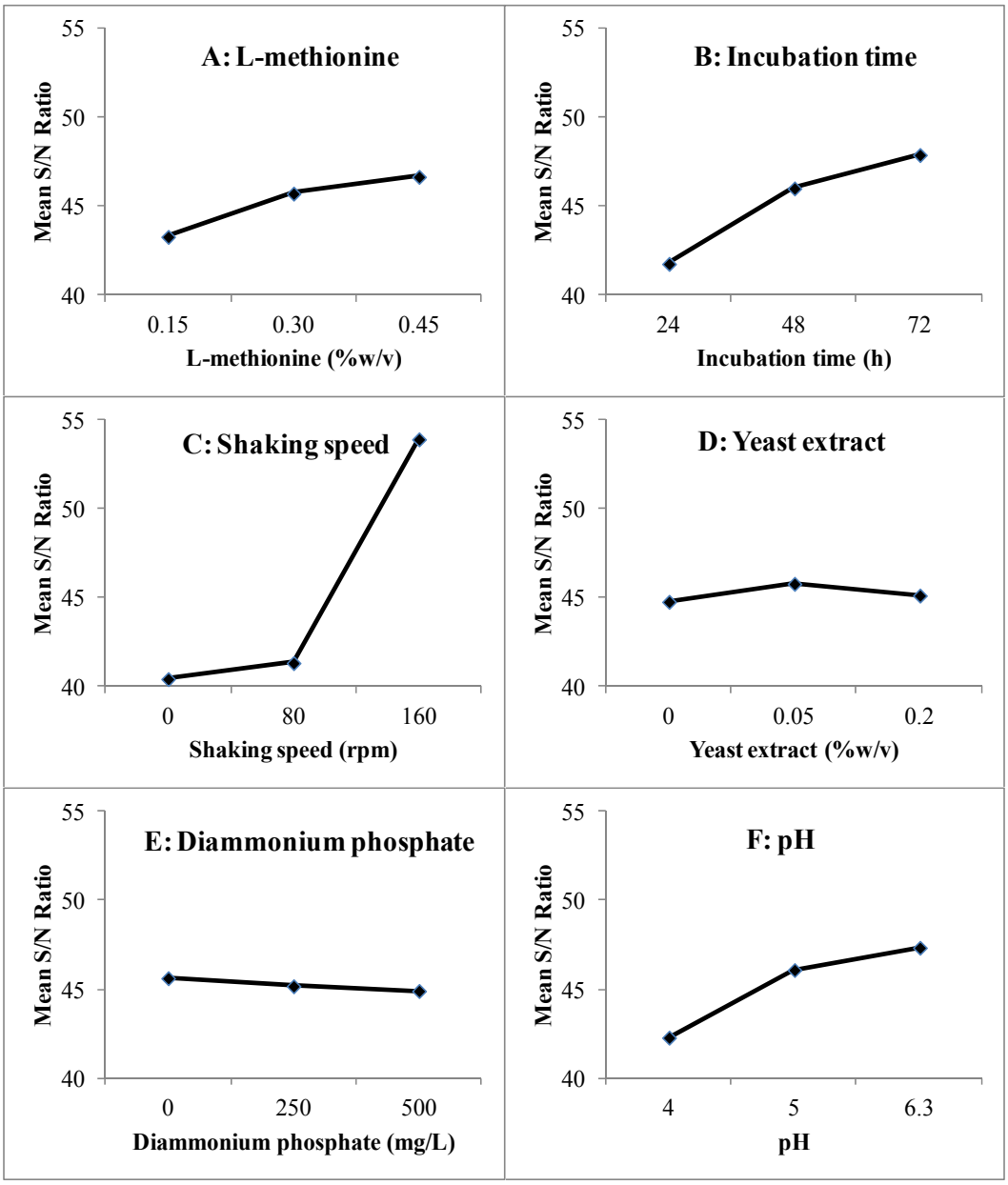

Figure 2. Main effects of parameters on the production of methionol (S/N ratio). The different data points in a single figure represent the average of S/N ratio at different levels of this studied parameter. The results were calculated based on the experimental data shown in Table 2

YE and DAP were introduced as growth and nutrient factors for *K. lactis*. Supplementation of YE was observed to cause a slight increase in S/N ratio at the low concentration of 0.05% (w/v) (Figure 2). This could be due to the presence of nutrients such as vitamins and amino acids in the YE that contributed to the growth of an increasing yeast population. However, as the amount of YE continued to increase to a concentration of 0.20% (w/v), the bioproduction of methionol decreased. Similar observations were reported in studies pertaining to *K. lactis* and *S. cerevisiae* fermentation and were attributed to nitrogen catabolite repression (Quek et al., 2011).

DAP was added as an inorganic nitrogen source for the nitrogen metabolism of yeast. However, it was found that the addition of DAP caused adverse effects on the bioproduction of methionol (Figure 2). A study similarly reported the decrease in the bioproduction of methionol and other volatile sulphur compounds when 300 mg/L of DAP was added into synthetic media containing *S. cerevisiae* (Rauhut, 2009). Another study also reported the inhibitory effect of ammonium chloride on VSFCs production by *Williopsis* yeast (Tan, Lee, Seow, Ong, & Liu, 2012). In this current study, the decreased production of methionol at higher levels of YE and DAP could be attributed to *K. lactis* assimilating other amino acids and nitrogenous substances present in the YE and the inorganic nitrogen from DAP instead of Met (Rauhut, 2009; Seow et al., 2010). This could thus result in decreased production of methionol since less Met was catabolized by the yeast. However, the effects of YE and DAP supplementation on methionol biosynthesis were not statistically significant. This may due to the narrow concentration range of supplementation being employed in this research, which was not sufficient to create a significant impact on the bioproduction of methionol.

The fermentation medium pH level is one of the key factors that affect the growth of yeast and bioproduction of methionol. From Figure 2, it can be seen that a higher pH value of 6.3 was observed to favour the bioproduction of methionol. This result was different from a previous study (Seow et al., 2010), where an optimum fermentation medium pH for the bioproduction of methionol was 5.0, implying that the enzymatic activity and growth of *K. lactis* was optimum under slight acidic conditions. Although both pH 5.0 and pH 6.3 are weak acidic fermentation conditions, one possible explanation for the difference could be the different experimental design methods used. In that previous study, the optimum fermentation conditions were obtained through single factor experimental design. In the present study, the Taguchi method, which allows different combinations of varying levels of parameters within every experimental run, was used. This could cause influential effects on the growth of *K. lactis*, hence giving rise to the difference in the optimum pH determined by these two studies.

Two interactive effects were studied: Met level and incubation time, and incubation time and shaking speed. Met is the precursor to the bioproduction of methionol, it was interesting to investigate whether incubation time and Met concentration have interactive effects on the formation of methionol. Similarly, it would also be important to observe the interactive effects between aeration and incubation time. The constructed interaction plots are shown in Figure 3. Interaction effects were observed to be non-significant, since there was no intersection between the lines, indicating that these parameters were independent of each other. In addition, the Taguchi method emphasizes that when an interactive effect is not statistically significant, the analysis of the optimum conditions would be solely on the main effects plots (Peace, 1993). Thus, the preferred levels for the fermentation conditions in this study were selected based on the main effects.

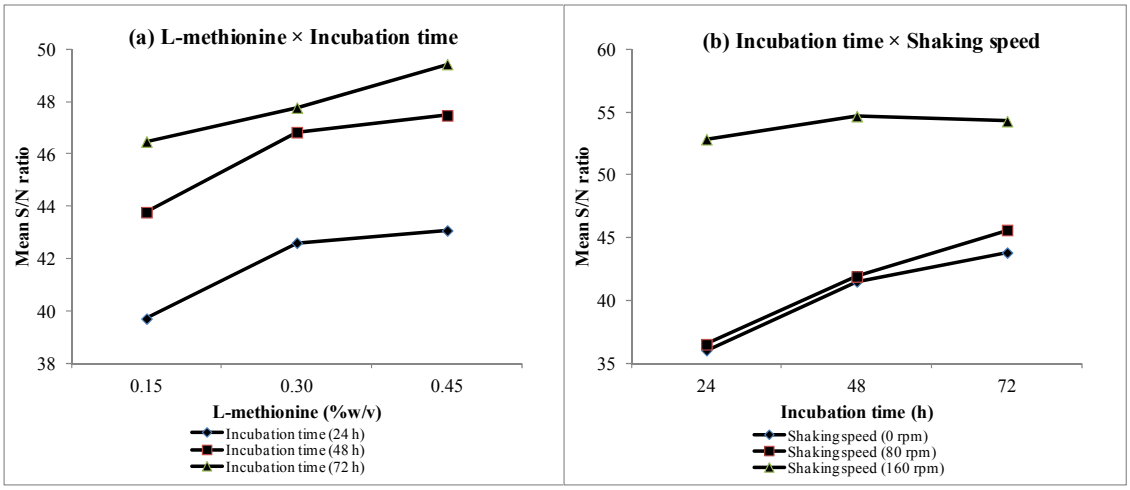

Figure 3. Interaction plots between parameters. (a) Met concentration and incubation time (A × B); (b) Incubation time and shaking speed (B × C). The interaction between studied two parameters was shown by three lines, and each line was drawn by fixing one of these two parameters at the same level

3.3 Confirmation Run and Scale-up Fermentation

Since the interest was based on a larger-the-better characteristic, the focus was to determine the levels of each significant parameter that resulted in the highest S/N ratio response. Cost-effective levels of the non-significant parameters such as YE could be implemented. Since 0.05% (w/v) of YE supplementation was economically acceptable, this level of incorporation was adopted into the confirmation run. According to the main effects plots shown in Figure 2, the optimum level of each parameter that gave the highest S/N ratio was chosen such as 0.45% (w/v) of Met, 72 h of incubation time, 160 rpm of shaking speed and pH 6.3. Less influential parameters such as YE and DAP, as well as weak interactions were not incorporated into the prediction equation to avoid an overestimate of the predicted S/N ratio. Although 72 h of fermentation gave the highest average S/N ratio, considering the economical aspects and the production efficiency, 48 h was selected as the optimum fermentation time since the difference between these predicted corresponding S/N ratios was not significant. For YE and DAP, the level produced highest S/N ratios according to main effect plots were selected as the optimum level which were 0.05% and 0 mg/L respectively. Therefore, the optimized fermentation conditions were determined as A_3 (0.45%), B_2 (48 h), C_3 (160 rpm), D_2 (0.05%), E_1 (0 mg/L) and F_3 (pH 6.3). Under the optimum conditions, the experimental S/N ratio obtained was 59.9 dB which was close to the predicted S/N ratio of 58.2 dB. And an average methionol concentration of 990.1 ± 49.7 µg/mL was achieved. A large-volume fermentation of 500 mL yielded an average methionol concentration of 766.0 ± 24.5 µg/mL. Using the Student's t-test, the bioproduction of methionol between 50 mL and 500 mL fermentation was found to be statistically different. This could be due to the difference in the dimensions of the glass bottles, where a 50 mL ferment subjected to shaking in a smaller bottle with lesser volume could result in more consistency during mixing.

Nevertheless, the experimental S/N ratios of the confirmation runs were found to be in good agreement with the predicted S/N ratio, having a low percentage deviation of 2.9%. When the actual S/N ratio was close to the predicted S/N ratio, the preferred levels of the parameters could be employed (Adnani et al., 2010; Kim, Choi, Choa, & Kim, 2007). In addition, while the study conducted by Seow et al. (2010) obtained a methionol yield of 130.0 µg/mL in 1-L aerated coconut cream fermented by *K. lactis* , the scale-up fermentation confirmation run in this study produced a much higher methionol concentration of 766.0 µg/mL. Similarly, the amount of methionol produced by *K. lactis* fermentation in our study was observed to be higher than that reported in another study (Lopez del Castillo-Lozano et al., 2007). Therefore, the results indicated that the Taguchi method was effective in optimizing the bioproduction of methionol by *K. lactis*. However, future study could be done in the area of applying the RSM approach to the optimization of methionol bioproduction. Therefore, an RSM approach should be proposed as a follow-up study and a comparison between optimized conditions by these two experimental design methods can then be made. The fermented coconut cream can be applied as flavour ingredients to food industry after sensory evaluation. On the other hand, methionol can be extracted from the coconut cream medium through solvent extraction or membrane separation methods, and the obtained methionol could be further applied in food industry as pure flavour compound.

4. Conclusion

In summary, the bioproduction of methionol by *K. lactis* in coconut cream supplemented with Met was successfully optimized using the Taguchi method. The main parameters that were observed to have a significant impact on methionol bioproduction were shaking speed, incubation time, pH and Met concentration. The experimental S/N ratio of the confirmation runs under the optimized conditions was 59.9 dB, which was in good agreement with the predicted S/N ratio of 58.2 dB. The amount of methionol produced in this study was also observed to be significantly higher compared to other studies. Therefore, the results suggest that the Taguchi method is effective in the optimization of methionol bioproduction by *K. lactis*. The application of RSM to optimize the bioproduction of methionol would be an ideal follow-up study to compare the results obtained by RSM and Taguchi method.

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