Ann Microbiol (2015) 65:197–205
DOI 10.1007/s13213-014-0850-y

ORIGINAL ARTICLE

Optimization of methionol bioproduction by *Saccharomyces cerevisiae* using response surface methodology

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Received: 14 October 2013 / Accepted: 13 February 2014 / Published online: 18 March 2014

Abstract Methionol (3-methylthio-1-propanol) is an important volatile sulphur-containing alcohol that may significantly impact food flavour. The purpose of this research is to investigate the bioproduction of methionol from L-methionine catabolism by *Saccharomyces cerevisiae* EC-1118. The biotransformation was carried out in coconut cream supplemented with L-methionine. Response surface methodology was applied for the optimization of fermentation conditions to achieve a high yield of methionol. A second-order polynomial model (R^2=0.912) was established based on the experimental data obtained in this study using multiple regression analysis. To obtain more accurate prediction results, a reduced quadratic model was obtained through backward elimination. Based on the reduced model, the optimal conditions for maximum methionol production were determined to be 0.30 % (w/v) of L-methionine, 0.10 % (w/v) of yeast extract and zero level of diammonium phosphate. Under these optimal conditions, a methionol concentration of 240.7±17.4 μg/mL was achieved. This experimental result was in close agreement with the predicted value of 243.5 μg/mL, indicating that this model was adequate. These results indicate that fermentation by *S. cerevisiae* in L-methionine-supplemented coconut cream medium is an effective method for the production of methionol. Large-scale fermentation trials are needed to provide valuable information for industrial production.

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Keywords Sulphur flavour · Methionol · L-methionine · *Saccharomyces cerevisiae* · Response surface methodology

Introduction

Volatile sulphur flavour compounds (VSFCs) are essential for the aromas of many food products and beverages such as cheese, beer and wine (Janssens et al. 1992). Methionol is an important VSFC that contributes significantly to the overall aroma profile of cheeses such as Cheddar and Camembert (López del Castillo-Lozano et al. 2007; Tan et al. 2012). It has a low odour threshold of 1 to 3 ppm and imparts cauliflower-like, meaty, savoury or toasted cheese flavour notes. The production of flavour compounds can be achieved by chemical synthesis, which can result in formation of racemic mixtures that require further downstream separation and purification (Janssens et al. 1992; Vandamme and Soetaert 2002). However, due to the increasing demand of consumers for natural products, alternative biotechnological methods are required (Hutkins 2006). Fermentation by microorganisms is one of the most important biotechnologies being applied for the production of natural flavour compounds (Vandamme and Soetaert 2002). Methionol is found to be one of the main VSFCs produced by yeast metabolism of L-methionine (Etschmann et al. 2008; Liu and Crow 2010). Biodegradation of the sulphur-carbon bond in L-methionine can lead to the formation of thiol intermediates and various VSFCs. Simplified pathways of L-methionine catabolism to produce VSFCs are presented in Fig. 1.

To obtain desirable flavour compounds, the bioconversion of specific substrates or precursors by fermentation has been gaining the interest of researchers in recent years. The sulphur-containing amino acid L-methionine was frequently applied as the precursor for the production of VSFCs. The reported yeasts that are able to biosynthesize VSFCs from L-

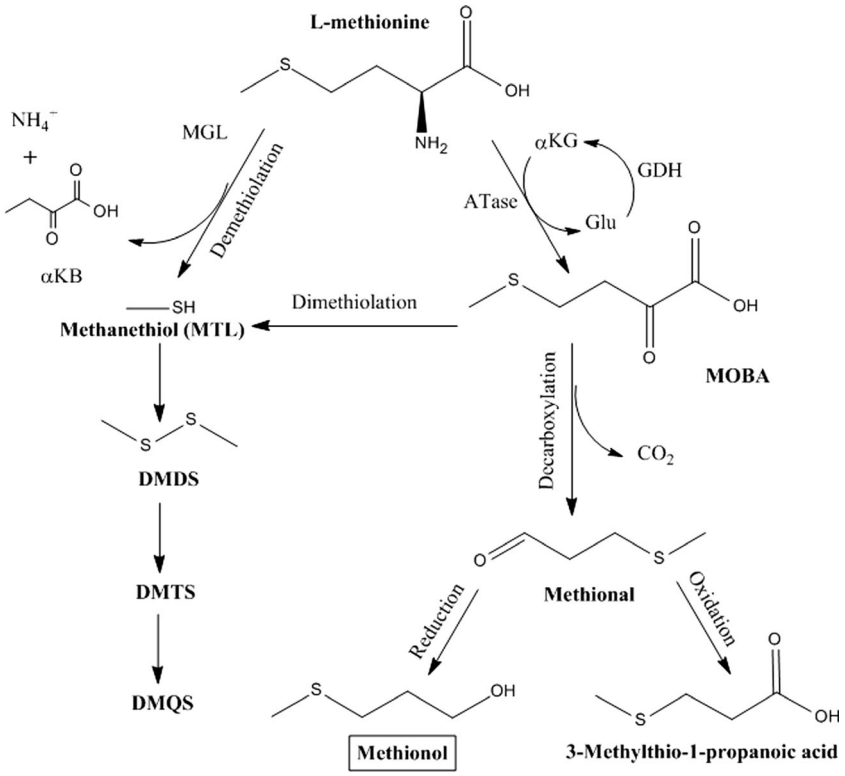

Fig. 1 Simplified pathways of L-methionine catabolism to produce methionol and VSFCs in numerous microorganisms, including yeasts and bacteria. VSFCs, volatile sulphur flavour compounds; Atase: Aminotransferase; α-KG: alpha-ketoglutarate; Glu: glutamate: GDH: glutamate dehydrogenase; MGL: methionine γ-lyase; MOBA: 4-methylthio-2-oxobutyric acid; DMDS: dimethyl disulfide; DMTS: dimethyl trisulfide; DMQS: dimethyl tetrasulfide. (modified after Yvon and Rijnen 2001; López del Castillo-Lozano et al. 2007; Rauhut 2009)

methionine include *Kluyveromyces lactis*, *Geotrichum candidum, Debaryomyces hansenii* and *Yarrowia lipolytica* (Spinnler et al. 2001; Yvon and Rijnen 2001; Kagkli et al. 2006; Cholet et al. 2008; Landaud et al. 2008). These yeasts are dairy yeasts that are normally used for cheese production. The application of these dairy yeasts to the production of methionol was rarely reported.

In addition to dairy yeasts, some wine yeasts such as *Saccharomyces cerevisiae* were also found to be capable of synthesizing VSFCs to modulate wine flavour (Swiegers and Pretorius 2007). *Saccharomyces cerevisiae* is known to metabolize L-methionine to methionol together with other VSFCs via the Ehrlich pathway (Perpète et al. 2006; Etschmann et al. 2008). However, the synthesis of methionol by *Saccharomyces cerevisiae* was mostly performed in synthetic media. Thus, it would be more relevant to investigate methionol production from L-methionine by *S. cerevisiae* in food matrices so that the fermented medium can be directly used as a flavouring ingredient.

In the current study, coconut cream was chosen as the fermentation medium due to its unique characteristics, such as low-cost, abundant availability in Southeast Asia, high fat content for better retention of flavour compounds, and nutrients for the growth of yeasts. To improve production of methionol in the coconut cream supplemented with L-methionine, it is essential to identify the optimal fermentation conditions. The factors that play important roles in methionol bioproduction include glucose concentration, L-methionine concentration and yeast extract level (Seow et al. 2010). To optimize the fermentation conditions, an effective and efficient experimental design is critical. The OVAT (one variable at a time) method is frequently applied, but this approach requires a large number of experiments, as more variables are involved.

Response surface methodology (RSM) is a multivariate statistic method that offers useful statistical information for improving and optimizing a process with only a small number of experiments. RSM is based on a series of statistical and mathematical methods that serve to describe the relationships between one or more responses and a number of independent variables (Bezerra et al. 2008; Sirisompong et al. 2011). As opposed to the OVAT approach, RSM is more efficient, and allows the interactive effects between variables to be assessed (Hasan et al. 2011). The Box-Behnken design (BBD) selects various points from the three-level factorial arrangements that allow first-order and second-order coefficients of the model to be estimated, significantly reducing the number of experiments (Bezerra et al. 2008).

Therefore, the objective of this study was to produce methionol through bioconversion of L-methionine by *S. cerevisiae* in L-methionine-supplemented coconut cream. Three nutritional factors, including L-methionine concentration, yeast extract level and diammonium phosphate level, were optimized using RSM with the BBD method,

because these were the significant factors that would affect L-methionie metabolism by yeasts, while other factors such as temperature and pH were not significant within the ranges tested (Cholet et al. 2008; López del Castillo-Lozano et al. 2007, Sirisompong et al. 2011).

Materials and methods

Yeast strain and pre-culture preparation

The yeast used was *S. cerevisiae* EC-1118 from Lallemand Inc., Ontario, Canada. Pure cultures were prepared in nutrient broth, which consisted of 0.25 % Bacto™ yeast extract (BD, Franklin Lakes, NJ, US), 0.25 % malt extract (Oxoid, Basingstoke, UK), 0.25 % peptone (Oxoid , Hampshire, England) and 2.0 % glucose (Glucolin®, GlaxoWellcome Ceylon Limited, Moratuwa, Sri Lanka). Initial pH of the nutrient broth was adjusted to pH 5.0 with 1.0 M hydrochloric acid (HCl) (Merck, Darmstadt, Germany). Two loopfuls of freeze-dried yeasts were added into 15 mL of sterilized nutrient broth and incubated at 25 ˚C for 28 h to obtain the pure yeast culture. Then 1.0 mL of the prepared pure yeast culture was added into 15 mL of UHT coconut cream (composition: 25.4 % fat, 4.6 % protein and 1.3 % carbohydrate; Kara, Fairteck Holding Pte. Ltd., Singapore) for propagation and adaptation before being inoculated into the actual experimental media for fermentation. After incubation at 25 ˚C for 72 h, the pre-culture was obtained with a cell count of around 10^7 CFU/mL.

Media preparation and fermentation

The fermentation medium was prepared aseptically by adding varied amounts of substrates and nutrients, including L-methionine, yeast extract and diammonium phosphate (DAP) (≥ 98 %, Sigma, Japan) into 100-mL blue-cap bottles containing 40 mL of coconut cream. Varied volumes of aqueous solutions of L-methionine with concentrations of 2.5 % and 3.0 % (w/v) were added into the 40 mL of UHT coconut cream to achieve final concentrations of 0.10 %, 0.20 % and 0.30 % (w/v). A stock solution of yeast extract (10.0 %, w/v) was added into the coconut cream in varied volumes to obtain three different concentrations of 0.10 %, 0.20 % and 0.30 % (w/v). DAP was applied as the yeast assimilable nitrogen (YAN) source, and 10.0 % w/v of DAP was spiked into the coconut cream in different volumes to get final concentrations of 0, 0.05 %, and 0.10 %, respectively. The initial pH of the experimental medium was adjusted to 5.0 with 1.0 M HCl. All solutions were prepared in deionized water and filter-sterilized (0.45 μm). The final volume consisting of the coconut cream and the added nutrients was adjusted to 50 mL with deionized water.

The fermentation by *S. cerevisiae* EC-1118 was performed by adding 1 % (v/v) of the pre-culture (initial 10^7 CFU/mL) into the aseptically prepared medium and incubating at 25 ˚C for 48 h. Two uninoculated controls were included: one consisting of only original coconut cream and the other of coconut cream supplemented with 0.30 % (w/v) of L-methionine. All the fermentations were carried out in duplicate and the mean of methionol concentrations was used as the response. The fermentations were stopped by adjusting the pH of fermentation medium to 2.5 with 1.0 M HCl. The samples were stored in 50-mL centrifuge tubes at −20 ˚C until analysis.

Sample analysis

Analysis of all samples was conducted with headspace (HS) solid-phase microextraction (SPME) coupled with gas chromatography (GC)-mass spectrometer (MS) and flame ionization detector (FID) (HS-SPME-GC-MS/FID). An 85 μm carboxen-polydimethylsiloxane (CAR-PDMS) fibre (Supelco, Bellefonte, PA, USA) was used for the adsorption of volatiles from the samples. Five millilitres of sample (diluted with 1 % of coconut cream water solution) were transferred to a 20-mL glass screw-thread vial (Agilent Technologies, CA, USA) for headspace extraction with the fibre. Extraction was conducted at 80 ˚C for 30 min in an automated shaking incubator with a shaking speed of 250 rpm. After adsorption, the fibre was placed in the injector port of an Agilent 6890 N network GC system (Palo Alto, CA, USA) for 2.5 min for thermal desorption into a DB-FFAP capillary column (60 m × 0.25 mm × 0.25 μm, Agilent, Woodbridge, USA). The carrier gas used was purified helium at a flow rate of 1.2 mL/ min. The initial oven temperature was set at 50 ˚C for 5 min, and was increased to 230 ˚C at 5 ˚C/min, and held for 10 min. The injector and detector temperatures were set at 250 ˚C. Splitless mode was applied.

Volatiles were identified based on comparison of their mass spectra with the WILEY database or standards. All analyses were conducted in duplicate. Quantitative analysis of methionol was performed through establishing standard calibration curves. Methionol (≥ 98 %, Sigma-Aldrich, St. Louis, MO, USA) standard solutions with different concentrations were prepared in 1 % (w/v) coconut cream solution.

Experimental design and statistical analysis

A three-factor, three-level BBD was applied to optimize the fermentation conditions, and the independent factors and levels studied are shown in Table 1. The parameters studied included L-methionine concentration (A, 0.10–

Table 1 Factors and their levels for Box-Behnken design

Parameter	Levels		
	−1	0	1
A: L-Methionine (% w/v)	0.10	0.20	0.30
B: Yeast extract (% w/v)	0.10	0.20	0.30
C: Diammonium phosphate (% w/v)	0	0.05	0.10

0.30 % w/v), yeast extract level (B, 0.10–0.30 % w/v) and DAP (C, 0–0.10 % w/v). A total of 17 experiments including five centre points were carried out according to the experimental design (Table 2). The experimental data obtained for model development were analysed with the Design Expert 6.0.10 (Stat–Ease, USA) software to conduct the response surface regression procedure to fit a second-order polynomial model Eq. (1):

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<j=2}^{3} \beta_{ij} X_i X_j \quad (1)$$

where y is the response (methionol concentration, μg/mL), β_0 is the intercept, β_i, β_{ii} and β_{ij} are the regression coefficients of the linear, quadratic and interactive terms, respectively. X_i and X_j represent the coded independent variables. The fitted polynomial equation can be expressed as surface plots for the visualisation of the relationships between the responses and the investigated parameters. Analysis of variance was conducted to evaluate the model developed and determine the factors significantly affecting the bioproduction of methionol. Optimal fermentation conditions were obtained with numerical optimisation function of the Design Expert software. Verification of the model developed was conducted through conducting six replicate fermentations under predicted optimal conditions. The experimental values obtained were compared to those predicted in order to determine the validity of the model. Results for the optimised methionol concentration were expressed as mean±standard deviation.

Statistic analysis was done using the Design Expert software. The second-order mathematic model was established based on the experimental data obtained. ANOVA and evaluation of the developed model were also performed by the Design Expert software.

Results and discussion

Model fitting

A total of 17 experiments were conducted to determine the optimum fermentation conditions, and the experimental results and predicted values are shown in Table 2, where the parameters including L-methionine concentration, yeast extract level and DAP level are represented by A, B and C, respectively. Based on the experimental data (Table 2), a

Table 2 The Box-Behnken design and actual, predicted methionol concentrations

Run	Parameters						Methionol (μg/mL)	
	Coded levels			Actual levels				
	A	B	C	A (% w/v)	B (% w/v)	C (% w/v)	Actual	Predicted [a]
1	−1	−1	0	0.10	0.10	0.05	152.2	138.6
2	1	−1	0	0.30	0.10	0.05	160.3	177.5
3	−1	1	0	0.10	0.30	0.05	87.1	105.9
4	1	1	0	0.30	0.30	0.05	145.9	144.8
5	−1	0	−1	0.10	0.20	0	155.5	151.4
6	1	0	−1	0.30	0.20	0	236.7	227.1
7	−1	0	1	0.10	0.20	0.10	134.4	148.3
8	1	0	1	0.30	0.20	0.10	141.9	150.4
9	0	−1	−1	0.20	0.10	0	195.5	205.6
10	0	1	−1	0.20	0.30	0	169.3	172.9
11	0	−1	1	0.20	0.10	0.10	173.1	165.7
12	0	1	1	0.20	0.30	0.10	148.0	133.0
13	0	0	0	0.20	0.20	0.05	137.4	141.7
14	0	0	0	0.20	0.20	0.05	131.6	141.7
15	0	0	0	0.20	0.20	0.05	147.0	141.7
16	0	0	0	0.20	0.20	0.05	159.2	141.7
17	0	0	0	0.20	0.20	0.05	154.6	141.7

[a] The predicted values were generated by the software based on reduced quadratic model (Eq. 3)

quadratic model was developed through regression analysis (Eq. 2):

$$y = 145.96 + 19.45A - 16.35B - 19.95C - 6.97A^2 - 2.62B^2 + 28.13C^2 + 12.67AB - 18.43AC + 0.27BC \quad (2)$$

Analyses of variance results are shown in Table 3. The significant model items were found to be L-methionine concentration (A), yeast extract level (B), DAP level (C), quadratic term for DAP level (C^2) and the interactive term of L-methionine concentration and DAP level (AC), all with p-values<0.05. The lack-of-fit was found to be not statistically significant for the model developed, which indicates that the model was adequate to demonstrate the relationships between responses and factors (Sirisompong et al. 2011; Zhao et al. 2011). The coefficient of determination (R^2) for the quadratic model was 0.912, indicating that it was a good fitting model and could adequately demonstrate the relationships between the response (methionol concentration, μg/mL) and the variables studied within the ranges. However, a higher R^2 does not necessarily mean that the regression model is better, as addition of a variable to the model always increases R^2, regardless of its statistical significance (Karazhiyan et al. 2011). Therefore, the R^2_{Adj} is more useful in the evaluation of model adequacy, as it takes into account the effect on R^2 due to the new variable. From Table 3, it can be seen that the R^2_{Adj} (0.798) was close to the R^2 value of 0.912, implying that the model was adequate. The coefficient of variation (CV) for the reduced model was found to be 8.95 %. The CV obtained indicates that variation in the mean value can be accepted, and thus the model could provide an adequate prediction. Therefore, all these results suggest that the model established could be employed for generating response surface graphs to show the effects of factors on the bioproduction of methionol.

Response surface plots

Response surface plots generated by the Design Expert software were used to study the effects of the parameters and their interactive effects on the bioproduction of methionol (Figs. 2, 3 and 4). These diagrams provide a method for the visualisation of the relationships of the responses at different experimental levels of each variable and the type of interactions between two variables. These figures show the effects of two factors on the response at one time while the other one factor was kept at coded level zero.

Figure 2 reveals the effects of L-methionine concentration and yeast extract level on the formation of methionol (DAP level fixed at 0.05 % w/v). From the response surface plot, it can be seen that the yield of methionol during fermentation increased upon increasing the L-methionine concentration. The highest methionol concentration was achieved with 0.30 % (w/v) of L-methionine and 0.10 % (w/v) of yeast extract. It was observed that more methionol was synthesised as more L-methionine was added into the fermentation medium, which was because that L-methionine was the important precursor for the production of methionol. It was reported elsewhere that the increment of L-methionine concentration can enhance the formation of methionol and other sulphur compounds (Moreira et al. 2002).

Figure 2 also shows that the bioconversion of L-methionine to methionol by yeast fermentation was inhibited by the yeast extract. As the yeast extract level increased from 0.10 % to 0.30 % (w/v), the production of methionol decreased. The highest amount of methionol was achieved at 0.10 % (w/v) of yeast extract, which was in agreement with a

Table 3 Coefficients of the quadratic model and analysis of variance result

Source	Sum of Squares	Degree of Freedom	Mean Square	F value	p value [b] Prob>F
Model [a]	13818.15	9	1535.35	8.01	0.006
A	3026.42	1	3026.42	15.80	0.005
B	2138.58	1	2138.58	11.16	0.012
C	3184.02	1	3184.02	16.62	0.005
A^2	204.40	1	204.40	1.07	0.336
B^2	28.85	1	28.85	0.15	0.710
C^2	3332.37	1	3332.37	17.40	0.004
AB	642.62	1	642.62	3.35	0.110
AC	1357.92	1	1357.92	7.09	0.032
BC	0.30	1	0.30	0.00	0.969
Residual	1340.93	7	191.56		
Lack of Fit	810.42	3	270.14	2.04	0.251
Pure Error	530.51	4	132.63		
Cor Total	15159.08 [c]	16 [d]			

[a] R^2 =0.912, R^2_{Adj}=0.798, C.V. = 8.95 %

[b] Confidence level of 95 %, $\alpha = 0.05$

[c] Cor total Sum of Squares: Sum of Squares total corrected for the mean

[d] Cor total Degree of Freedom: Degree of Freedom total corrected for the mean, number of runs minus one

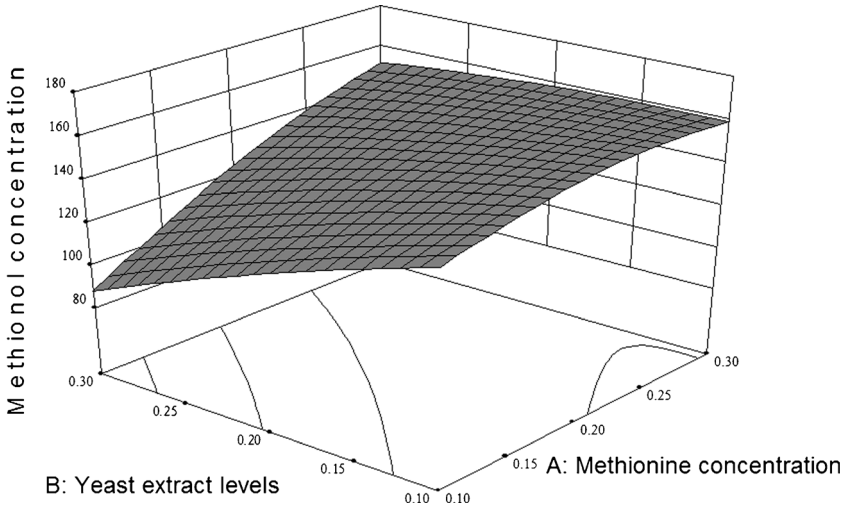

Fig. 2 Effect of (A) L-methionine concentration (% w/v) and (B) yeast extract level (% w/v) on the bioproduction of methionol (μg/mL)

previous study (Quek et al. 2011) on the production of methionol in soymilk medium. That may be because as more yeast extract was added, more YAN was introduced into the fermentation medium, which could reduce the production of methionol (Carrau et al. 2008; Rauhut 2009). The similar observation was also reported in previous studies where an increase in yeast extract levels beyond 0.10 % (w/v) led to a decrease in methionol production by different yeasts (Seow et al. 2010; Quek et al. 2011). Moreover, the adverse effect of yeast extract on the bioproduction of methionol became much more prominent at low concentrations of L-methionine.

The interactive effects of L-methionine concentration and DAP level on the yield of methionol are shown in Fig. 3 (yeast extract level was fixed at 0.20 % w/v). The highest concentration of methionol was achieved at 0.30 % (w/v) of L-methionine and zero DAP level. Methionol concentration was found to increase with the increase in L-methionine concentration at lower DAP concentrations (less than 0.05 % w/v). Interestingly, we found that the DAP level affected the bioproduction of methionol in different ways as different amounts of L-methionine were added into the

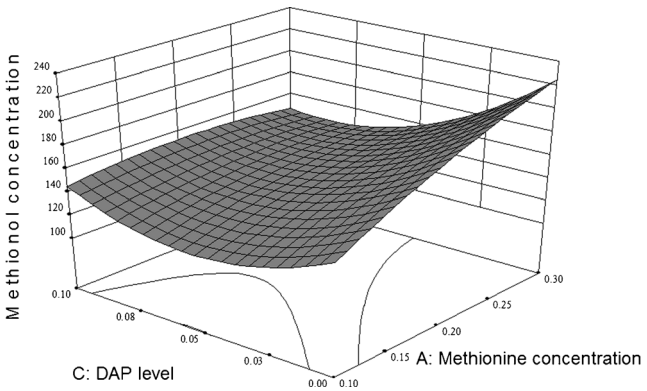

Fig. 3 Effect of (A) L-methionine concentration (% w/v) and (C) diammonium phosphate (DAP) level (% w/v) on the bioproduction of methionol (μg/mL)

fermentation medium. With 0.10 % (w/v) of L-methionine, methionol concentration was reduced as the DAP level was decreased from 0.10 % to about 0.05 %, and it increased as the DAP level further decreased to 0. However, this fluctuation of methionol concentration was not statistically significant, especially when a high amount of L-methionine was added.

With 0.30 % (w/v) of L-methionine, a huge reduction in methionol bioproduction was observed as more DAP was added. This clearly indicates an inhibitory effect of DAP on methionol production, which was especially significant at high L-methionine concentrations. This may be because the addition of DAP as one type of YAN reduced the need of yeast cells to metabolize L-methionine for growth, hence leading to decreased bioconversion of L-methionine into methionol. The inhibitory effect of nitrogen supplementation on methionol production by *S. cerevisiae* was similar to that reported previously (Tan et al. 2012). For comparison, a higher initial ammonia concentration also decreased formation of higher alcohols and methionol by *S. cerevisiae* (Hernández-Orte et al. 2005; Hernández-Orte et al. 2006).

Figure 4 shows the effects of yeast extract and DAP levels on the bioproduction of methionol, while L-methionine concentration was kept at 0.20 % (w/v). Methionol concentration was found to decrease linearly with the increase of yeast extract level. Similarly, methionol concentration decreased upon increasing DAP level up to about 0.05 % (w/v). Therefore, high concentrations of yeast extract and DAP were found to inhibit the formation of methionol. However, as is apparent in Fig. 4, the adverse effects on methionol production brought about by the addition of yeast extract were not as significant as that of DAP. This was because DAP contributed more YAN as compared to yeast extract. The later plateau-off in the production of methionol suggests that DAP concentrations ranging from 0.05 to 0.10 % (w/v) exerted similar inhibitory effects on methionol production. Methionol concentration was found to be higher at 0.10 % (w/v) yeast extract

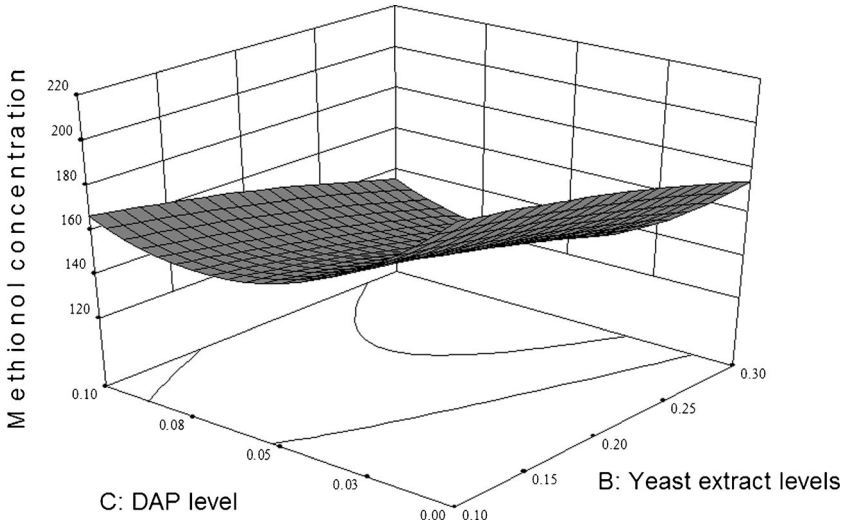

Fig. 4 Effect of (B) yeast extract level (% w/v) and (C) diammonium phosphate (DAP) level (% w/v) on the bioproduction of methionol (μg/mL)

and zero DAP. It was noted that the effect of yeast extract on methionol yield was conserved at all DAP levels, and the same went for DAP level. This indicates that there was no significant interactive effect between these two factors within the ranges studied, and thus, increasing the concentrations of either DAP or yeast extract would lead to a decrease in methionol production.

Attaining optimal conditions and verification

Since the full quadratic model developed (Eq. 2) includes not only significant terms, but also non-significant terms such as AB, BC, A^2 and B^2, to obtain more accurate predication results, a reduced model was obtained by removing non-significant terms through backward elimination (Eq. 3):

$$y = 141.70 + 19.45A - 16.35B - 19.95C + 27.60C^2 - 18.42AC \quad (3)$$

This reduced model was also evaluated, and the ANOVA result (data not shown) indicates that this model is adequate for the prediction of optimal fermentation conditions. To obtain optimal levels of each parameter, numerical optimization and point prediction functions of the Design Expert software were employed based on the reduced model (Eq. 3). The predicted values for experimental runs are shown in Table 2. The optimal conditions for methionol production

Table 4 Volatile sulphur flavour compounds (VSFCs) identified in fermented and control samples

VSFCs	FID Peak Area (×10^6) [a]			
	Sensory description	Coconut cream+Met fermented	Coconut cream control	Coconut cream+Met uninoculated
Methionol	Sweet, potato [b]	263.61	nd [g]	nd
Methional	Boiled potato-like [c]	2.91	nd	18.5
3-(Methylthio)-propionic acid	Chocolate, roasted [d]	0.84	nd	nd
3-(Methylthio)-propyl acetate	Fatty, ester [e]	35.62	nd	nd
3-(Methylthio)-propionic acid ethyl ester	Metallic, sulphur aroma [f]	5.59	nd	nd
1,3-Oxathiane	Not reported	0.68	nd	nd
2-Methyl-tetrahydrothiophen-3-one	Metallic, natural gas odour [f]	0.10	nd	nd
Dimethyl disulphide	Onion, cabbage [b]	nd	nd	1.34

[a] Average FID peak areas of volatile compounds in 5 mL of undiluted sample

[b] www.flavornet.org

[c] Yvon and Rijnen (Yvon and Rijnen 2001)

[d] Pripis-Nicolau et al. (Pripis-Nicolau et al. 2004)

[e] www.thegoodscentscompany.com

[f] Moreira et al. (Moreira et al. 2002)

[g] Not detected

were found to be 0.30 % (w/v) of L-methionine concentration, 0.10 % (w/v) of yeast extract level and zero DAP level. The observed average experimental value of methionol based on six replicate fermentations conducted under the optimized conditions was 240.7±17.4 μg/mL, which was in close agreement with the predicted methionol value of 243.5 μg/mL. Moreover, the obtained experimental value was also well within the confidence and predicted intervals at 95 % confidence level specified by the Design Expert software (data not shown). Therefore, the results demonstrated the validity of the model for the prediction of optimal fermentation conditions.

Qualitative analysis of VSFCs

A total of eight VSFCs in the undiluted fermented and control samples were detected and are shown in Table 4. No VSFCs were found in the control (original coconut cream only). Among these VSFCs, only dimethyl disulphide (DMDS) was not found in the fermented samples, while methional and DMDS were detected in the uninoculated control samples containing added L-methionine. The presence of DMDS and methional in the uninoculated control containing added L-methionine indicated occurrence of Strecker degradation (Tan et al. 2012). Methional was detected in the fermented samples, but its level was lower compared to that in the L-methionine-supplemented controls.

In addition to chemical degradation, methional can also be formed via the Ehrlich pathway of L-methionine catabolism (López del Castillo-Lozano et al. 2007). Methional, methionol and methanethiol resulted from L-methionine catabolism by yeasts act as precursors for other VSFCs, including methionic acids, 3-(methylthio)-propyl acetate (3-MTPA) and 3-(methylthio)-propionic acid ethyl ester (3-MTPE) (López del Castillo-Lozano et al. 2007; Tan et al. 2012). Methionol and 3-MTPA were two major VSFCs in the fermented samples.

1,3-Oxathiane and 2-methyl-tetrahydrothiophen-3-one were only found in fermented samples. The presence of these two VSFCs has been reported in L-methionine and L-methionine-L-cysteine mixture supplemented culture media after being fermented with cheese-ripening yeasts including strains of *S. cerevisiae*, *K. lactis* and *D. hansenii* (López del Castillo-Lozano et al. 2007). Production of thiophene compounds by ascomycetous yeasts such as *K. lactis, S. cerevisiae, D. hansenii* and *Y. lipolytica* had not been reported before (López del Castillo-Lozano et al. 2007). Further studies are needed to better understand the production of these VSFCs by yeasts.

Conclusions

Methionol bioproduction by *S. cerevisiae* EC-1118 was optimized with RSM based on the three-factor, three-level BBD method. L-Methionine concentration, yeast extract and nitrogen levels were significant in affecting methionol bioproduction. The results indicate that fermentation by *S. cerevisiae* in L-methionine-supplemented coconut cream is effective for producing methionol. The fermented product may be developed as a direct bioflavouring ingredient ("coconut-based sulphur flavour concentrate") in food applications such as processed cheese, enzyme-modified cheese, imitation cheese, soups and sauces.

Conflict of Interest Statement The authors declare that they have no conflict of interest.

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