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Optimization of Single Cell Protein Production by *Candida utilis* Using Juice Extracted from Pineapple Waste through Response Surface Methodology

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ABSTRACT

Response surface methodology was applied to optimize protein content in *Candida utilis* grown in pineapple waste medium. A three-level full factorial design was used to develop a quantitative interpretation of mathematical models between the two variables studied, inoculum size 2.0-10.0% (v/v) and total soluble solids in medium (1°-5° Brix) at 30 h fermentation time. Yeast cells were harvested, ruptured mechanically and the soluble extract was freeze-dried for determination of protein, vitamin-B, 5'-ribonucleotide and total sugar content. Maximum protein content in the yeast 66.61% (w/w) was obtained from the predicted optimum inoculum size of 7.83% (v/v) and Brix level of 3.02°. Highest level of biomass, vitamin-B, 5'-ribonucleotide and total sugar content within the experimental region increased 216.8%, 17.5%, 38.0% and 60.8% respectively after optimization. A verification experiment, conducted at optimized protein content conditions produced values that were close to the predicted values, indicating the reliability of the model used.

Key words: Response Surface Methodology; RSM; yeast extract; pineapple waste; *Candida utilis*

INTRODUCTION

More than six million hectares of land in Malaysia are utilized for major crops such as oil palm, rubber, paddy, pineapple, coconut and cocoa. Agricultural biomass contributes up to 14% of energy consumption which reaches 340 boe annually. Despite this, only 24.5% of the total agricultural biomass is fully utilized for energy consumption and the rest are left as wastes (Ministry of Agriculture, 2002). Pineapple waste has been found to contain up to 6.14% of carbohydrate, minerals especially magnesium and 0.6% of crude protein (Hutagalung, 2002), thus undoubtedly a valuable fermentation substrate for both single cell protein (SCP) and metabolites production. *C. utilis* and *Hansenula anomala* showed encouraging growth with biomass containing high protein content while *S. cerevisiae* showed high productivity of ethanol (Nigam, 1999). Hence, with more than 11000 hectares of land in Malaysia currently planted with pineapples which generate 40-65 tonnes of waste per hectare, economical fermentation medium could be produced for the production of high quality yeast extract (Agrolink, 2002).Yeast extract (YE) has been reported as a good source of supplement for protein deficient diet. Protein content from dried yeast biomass may range from 45-50% w/w and over 60% w/w is in YE, thus making it an important SCP source (Sgarbieri *et. al.*, 1999). Yeast cell matter is particularly rich in most of the B-group vitamins ranging from 5.53 to 60.70 mg kg^{-1} dried cell and therefore constitutes a potential source of enrichment for vitamin B-deficient diets as well (Wang *et. al.*, 2000). YE from *C. utilis* was also found to contain high amount of glucose and total sugar content (Liong *et. al.*, 2002).

The utilization of Response Surface Methodology (RSM) in the field of fermentation is limited to the optimization of microbial growth (Vazquez and Martin, 1998), production of metabolites such as astaxanthin (Ramirez *et al.*, 2001) and microbiological media improvement (Li *et. al.*, 2002). The main objective of the present study was to optimize medium composition for improvement of protein content in *C. utilis* using response surface methodology.

MATERIALS AND METHODS

Microorganism and culture conditions

C. utilis 1017 was obtained from the Culture Collection Center of Universiti Teknologi Mara, Shah Alam, Malaysia. The freeze-dried cultures were reactivated, maintained on YEPG (Yeast extract, peptone, glucose) agar slants at 4°C, and restreaked to YEPG agar plates periodically. A loopful of *C. utilis* was inoculated into 50 ml of pineapple waste broth, incubated at 30 °C for 24 h on a rotary shaker at 150 rpm to obtain 10^6 cells/ml inoculum. An amount of 2.0-10.0 % (v/v) of this inoculum was inoculated into a 2 L fermentor (B. Braun Biolab, Germany) containing 1.5 L pineapple waste medium and cultivated at 30 °C for 30 h at 500 rpm. Agitation

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was provided by a turbine impeller and air was delivered at 11.0 ml min^{-1}.

Pineapple waste medium

Pipeapple waste consisting of peels, cores and unwanted parts of pineapples, was obtained from local fruit stalls, washed prior to cutting into smaller pieces (1-2 cm) before adding with distilled water (1:1 (w/v)). The extract was obtained through heat treatment using autoclave for 30 minutes at 121°C and 15 p.s.i. pineapple waste extract was diluted to obtain 1-5 °Brix, filtered using filter paper (Whatman No.1) and glass microfiber filter (Whatman GF/C). pH was adjusted to 4.0 with HCl before autoclaving (15 minutes, 121°C, 15 p.s.i).

Extraction of protein from yeast cells

The method was modified from Catley (1988). Cells were harvested by centrifugation and dried at 30-35 °C in an oven. The dried cells were mixed with glass beads of 0.45-0.50 mm in diameter (Sigma, USA) and acetate buffer (pH 5.0, 4°C) at a ratio of 1:4:4 (w:w:v) respectively. Mechanical rupturing of yeast cells was achieved through vortexing of the mixture for 30 seconds and storing in ice-bath for 1 minute. This step was repeated till cells were detected as ruptured through microscopic examination. After centrifugation, crude protein was freeze-dried (-50°C, vacuum 100 μHg, 24 hours).

Analytical methods

Dry cell weight was determined gravimetrically, the cells being harvested by centrifugation at 12000 rpm for 10 minutes in microcentrifuge tubes, washed twice with distilled water and recentrifuged before drying at 30-35°C until a constant weight was obtained. Protein content was determined by the Biuret method (Robinson and Hodgen, 1940). Total sugar content was determined by the phenol-sulfuric acid method (Dubois *et al.*, 1956) and the reducing sugars content by the Nelson-Somogyi method (Somogyi, 1952) and carboxylic acid content by the metavanadate method (Dima and Ghimicescu, 1977).

5'-ribonucleotide assay

5'-ribonucleotide was determined by using high performance liquid chromatography (HPLC) method (Yang *et. al.*, 2001) with some modifications. Sample (0.5 g) was mixed with 25 ml distilled-deionized water and heated at boiling temperature for 1 min before being cooled and centrifuged at 3500 rpm for 30 min. Extraction was repeated with 20 ml distilled-deionized water. Both extracts were combined and rotary-evaporated to achieve a final volume of 10 ml. Concentrated extract was filtered (0.45 μm) before injecting into the HPLC (Waters, Milford).

Separation was performed with a Nova-Pak C_{18} column (3.9 x 150 mm, Milford) and detected by Waters UV detector (996 Photodiode Array Detector, Milford) at 254 nm. The mobile phase was 0.5 M KH_2PO_4/H_3PO_4 (pH 4.0) at a flow rate of 1 ml min^{-1}. 1 mM 5'-Inosine monophosphate (Sigma, USA) and 5'-Guanosine monophosphate (Sigma, USA) were used as standards.

Vitamin-B complex assay

Niacin, pyridoxine, riboflavin and thiamin were determined by using the HPLC method (Anon USP, 1995) with some modifications. Sample (1.0 g) was dissolved in 25 ml diluting solution consisting of water:acetonitrile:glacial acetic acid at a ratio of 94:5:1 (v:v:v). The mixture was vortexed for 30 s and then heated at 65-70 °C for 5 min in the water bath. Vortex-mixing and heating were repeated twice, before the mixture was cooled to room temperature and filtered (Whatman, 0.45 μm) prior to analysis by HPLC. Standards were prepared by mixing 80 mg niacin, 20 mg pyridoxine, 20 mg riboflavin and 20 mg thiamin in 180 ml diluting solution. The mixture was vortexed, heated in water bath (65-70°C) for 5 min, cooled to room temperature and was diluted with distilled water to a final volume of 200 ml before filtration. Standards were kept at -20°C and refreshed every month.

The HPLC system was the same as for 5'-ribonucleotide assay. The mobile phase was water:methanol:glacial acetic acid at the ratio of 73:26:1 with addition of 1.4 mg ml^{-1} sodium-1-hexanesulfonate. Flow rate was 1 ml min^{-1} and UV detection was performed at 280 nm. Each sample was quantified by comparison to the vitamin-B standards.

RSM Experimental Design

The optimum operational conditions for the batch fermentation of *C. utilis* using pineapple waste medium were determined by means of RSM. The dependant variables chosen were protein content in yeast, yeast growth, total sugar content, vitamin-B content and 5'-ribonucleotide content in yeast. The independent variables used for this study were inoculum size and total soluble solids of substrate. The units and the coded levels of the independent variables are shown in Table 1. Experimental data are mean of triplicate determinations.

Table 1: Experimental Design Levels for independent Variables in RSM

Independent variables	Symbols		Levels		
	Uncoded	Coded[a]	-1	0	1
Inoculum size (% v/v)	Ino	X_1	2	6	10
Substrate Brix level (°)	Brix	X_2	1	3	5

[a] X_1: (Ino - 6)/4; X_2: (Brix - 3)/2

Statistical Analysis

A three-level full factorial design was used to develop a quantitative interpretation of mathematical models between the two variables studied, inoculum size 2.0-10.0% (v/v) and

substrate Brix level (1°-5°). These analysis were performed using the Design Expert version 5.07 software (Stat-Ease Corp., Minneapolis).

RESULTS AND DISCUSSION

The 3-level full factorial experimental design matrix and the results obtained for all dependent variables are shown in Table 2. These results were used to develop response surface models based on the following equation: $Y_i = c_o + c_1X_1 + c_2X_2 + c_3X_1^2 + c_4X_2^2 + c_5X_1X_2$ where Y_i (i: 1-5) is the dependent variable, X_1 and X_2 are the independent variables, and c_1-c_5 are the coefficients obtained by multiple regression of the experimental data.

Table 2: Three Level Full Factorial Design Matrix and Results of Dependent Variables.

Experimental runs	Independent variables		Dependent variables				
	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5
1	-1	-1	40.51	4.8	15.77	93.30	14.65
2	-1	0	55.18	8.5	17.26	88.09	14.1
3	-1	1	37.98	13.5	18.92	79.40	11.19
4	0	-1	55.44	7.2	18.30	98.72	15.52
5	0	1	53.69	14.8	27.14	94.24	17.77
6	0	0	62.26	11.0	22.63	109.97	17.28
7	0	0	65.95	11.3	21.85	117.25	17.77
8	0	0	61.87	10.7	23.87	111.58	17.12
9	0	0	62.71	11.8	21.68	106.39	17.48
10	0	0	71.36	10.5	21.36	94.24	16.96
11	1	-1	52.27	5.4	12.99	51.87	13.92
12	1	0	63.63	11.1	17.68	43.85	19.01
13	1	1	54.71	14.8	18.93	49.01	19.75

X_1: Inoculum size (%v/v); X_2: Substrate Brix level; Y_1: Yeast extract protein content (% w/w); Y_2: Yeast biomass (g/l); Y_3 : Yeast extract total sugar content (%w/w); Y_4: Yeast extract vitamin-B content (mg/100g sample); Y_5: Yeast extract 5'-ribonucleotide content (mg/g sample). Experiments were conducted in a random order. Experimental results are averages of triplicate determinations.

Optimization of protein content in yeast

Protein content in yeast varied within the range of 37.98-71.36% (w/w). ANOVA results (Table 3) showed that the second-order model corresponded well with experimental data. Lack-of-fit did not show any significance. Independent variable inoculum size had greater influence towards protein content compared to substrate Brix level, and the influence was found to be significant at alpha value of 0.05. Best explanatory equation for the response surface is shown in Table 4. Interaction effects of both independent variables were lower compared to individual effect of inoculum size. The R^2 was satisfactory with 7.44% total variation not explained by the model.

Response surface (Figure 1) shows that bigger inoculum size 10.0% (v/v) produced higher protein content compared to smaller inoculum size 2.0% (v/v). Intermediate Brix level (3°) produced highest protein content for both inoculum size 2.0% (v/v) and 10.0% (v/v). Protein content in yeast was predicted to be maximum 66.61% (w/w) at inoculum size of 7.83% (v/v) and Brix 3.02°. This marked an increase of 63.34% compared to non-optimized fermentation 40.51% (w/w) at inoculum size of 2.0% (v/v) and Brix level 1°. Optimized protein content was comparable to YE of *S. cerevisiae* 61.5% (w/w) from the ethanol industries (Sgarbieri *et. al.*, 1999).

Table 3: Analysis of Variance for Dependent variable

Source	Sum of squares	DF	Mean square	F-value	Probability>F
Regression					
Linear	228.10	2	114.05	1.32	0.3097
Quadratic	782.10	3	260.70	22.46	0.0006[a]
Cubic	12.16	2	6.08	0.44	0.6669
Residual	69.09	5	13.82	-	-
Model:	1010.20	5	202.04	17.41	0.0008[a]
Lack-of-Fit	17.47	3	5.82	0.37	0.7828
Pure error	63.77	4	15.94	-	-
Factor:	**Coefficient estimate**	**DF**	**Standard error**	**t for H_o coefficient=0**	**Probability>t**
Inoculum size (% v/v)	6.16	1	1.39	4.43	0.0031[a]
Brix level (°)	-0.31	1	1.39	-0.22	0.8326
R^2= 0.9256					

Protein content of YE(Y_1)
DF: degree of freedom; [a]Significance at the 5% level

Table 4: Predicted equations for dependent variables

Dependent variable	Best explanatory equation
Protein content	$Y_1 = 65.21 + 6.16X_1 - 0.31X_2 - 6.76X_1^2 - 11.60X_2^2 + 1.24X_1X_2$
Yeast growth	$Y_2 = 11.07 + 0.76X_1 + 4.27X_2 - 1.31X_1^2 - 0.13X_2^2 + 0.19X_1X_2$
Vitamin-B content	$Y_3 = 105.98 - 19.34X_1 - 3.54X_2 - 35.24X_1^2 - 4.73X_2^2 + 2.76X_1X_2$
5'-ribonucleotide content	$Y_4 = 17.50 + 2.02X_1 + 0.77X_2 - 1.09X_1^2 - 1.31X_2^2 + 2.32X_1X_2$
Total sugar ontent	$Y_5 = 22.62 - 0.39X_1 + 2.99X_2 - 5.52X_1^2 - 0.27X_2^2 + 0.70X_1X_2$

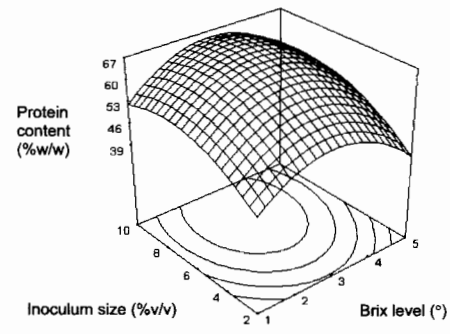

Figure 1: Response surface for dependent variable, protein content of YE

In medium containing glucose, yeasts were found capable of accumulating succinate intracellularly due to diffusion of carboxylic acid from medium. Succinic acid bridges synthesis of important cell components including

intracell protein (Kratochvilova, 1990). Thus, utilization patterns and content of carboxylic acid in yeast cell were studied in terms of succinic acid. Pineapple waste medium at Brix levels 1°, 3° and 5° was found to consist of 0.091 mg/ml, 0.125 mg/ml and 0.199 mg/ml succinic acid respectively. Utilization patterns of succinic acid is shown in Figure 2. Utilization was found to increase with the increment of both inoculum size and substrate Brix level, with increment of the latter producing greater utilization increment. This observation suggests that the reducing sugar content for cell metabolism was sufficient. Cassio and Leao (1993) found that under such conditions, associated form of dicarboxylic acid was capable of entering cells through simple diffusion, thus increasing utilization. This was also supported by increased carboxylic acid needs to support metabolism of the Kreb cycle at higher cell density (Kratochvilova, 1990).

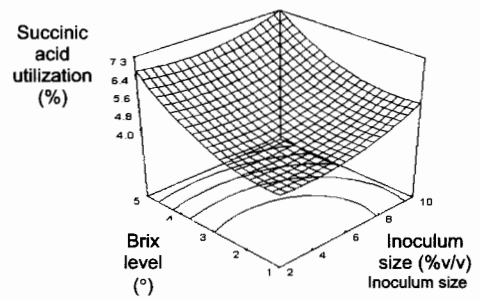

Figure 2: Response surface for succinic acid utilization patterns

Response surface of succinic acid content in yeast cell (Figure 3) shows that variation patterns across different Brix levels were small as compared to inoculum size. Similar to protein content in yeast cell, succinic acid content also increased with the increment of inoculum size. At certain glucose content, carboxylic acid was found to compete with malic acid for the same transportation pathway in yeast cell (Cassio and Leao, 1993). Thus, results obtained here suggested that the increment of inoculum size reduced these glucose effects of pathways competition. This may contribute to the increment of succinic acid content in yeast cell and thus increased protein content in yeast cell

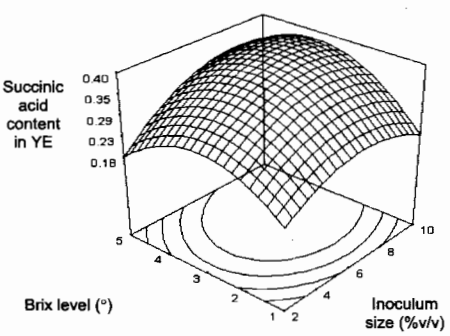

Figure 3: Response surface for succinic acid content in YE

Evaluation of yeast growth and total sugar content in yeast cell

Yeast growth as measured by means of cell concentration showed that it varied within the range of 4.8-14.8 g/L and total sugar content in yeast cell varied within the range 12.99-27.14% (w/w). ANOVA results (Table 5 and 6) shows that experimental data corresponded well with the second-order model. Lack-of-fit test was not significant. Independent variable Brix level had stronger influence towards yeast growth as compared to inoculum size. Interaction term of independent variables had less effect towards yeast growth compared to individual effects of Brix level (Table 4).

Table 5: Analysis of Variance for Dependent Variable Yeast Biomass (Y_2)

Source	Sum of squares	DF	Mean square	F-value	Probability>F
Regression:					
Linear	112.68	2	56.34	63.75	<0.0001[a]
Quadratic	6.17	3	2.06	5.40	0.0306[a]
Cubic	1.61	2	0.80	3.79	0.0996
Residual	1.06	5	0.21	-	-
Model:	118.85	5	23.77	62.44	<0.0001[a]
Lack-of-Fit	1.61	3	0.54	2.04	0.2504
Pure error	1.05	4	0.26	-	-
Factor:	**Coefficient estimate**	**DF**	**Standard error**	**t for H_o coefficient=0**	**Probability>t**
Inoculum size (% v/v)	0.76	1	0.25	3.01	0.0196[a]
Brix level (°)	4.27	1	0.25	16.94	<0.0001[a]
R^2= 0.9781					

DF: degree of freedom; [a]Significance at the 5% level

Table 6: Analysis of Variance for Dependent Variable Total Sugar Content of YE (Y_3)

Source	Sum of squares	DF	Mean square	F-value	Probability>F
Regression:					
Linear	2320.18	2	1160.09	2.33	0.1477
Quadratic	4524.84	3	1508.28	23.24	0.0005[a]
Cubic	28.20	2	14.10	0.17	0.8520
Residual	426.05	5	85.21	-	-
Model:	6845.02	5	1369.00	21.10	0.0004[a]
Lack-of-Fit	160.13	3	53.38	0.73	0.5874
Pure error	294.12	4	73.53	-	-
Factor:	**Coefficient estimate**	**DF**	**Standard error**	**t for H_o coefficient=0**	**Probability>t**
Inoculum size (% v/v)	-19.34	1	3.29	-5.88	0.0006[a]
Brix level (°)	-3.54	1	3.29	-1.08	0.3174
R^2= 0.9378					

DF: degree of freedom; [a]Significance at the 5% level

Response surface (Figure 4) clearly indicates that yeast growth may further increase with the increment of substrate Brix level, with less change to be achieved from single effect of inoculum size. Predicted maximum yeast growth (15.25 g/L) was obtained from the combination of Brix 5° and inoculum size 6.0% (v/v), showing an increment of 216.79% compared to non-optimized fermentation process (4.80 g/L). Predicted yeast growth from optimized fermentation (Brix 3.02°, inoculum 7.83% (v/v) was also high (11.19 g/L) with an increment of 133.13% compared to non-optimized fermentation process.

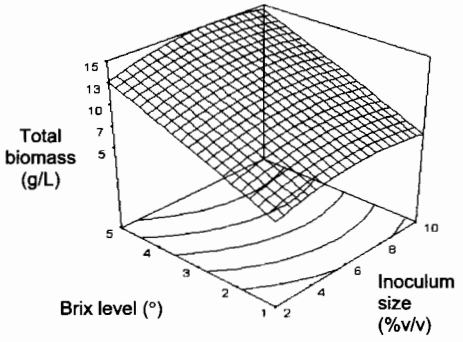

Figure 4: Response surface for dependent variable, growth of *C. Utilis*

Response surface (Figure 5) indicates that total sugar content in yeast cell may be increased with the increment of Brix level. Maximum sugar content within experimental region 25.35% (w/w) was predicted at inoculum size 6.11% (v/v) and Brix 5°. This marks an increase of 60.76% compared to before optimization 15.77% (w/w). Sugar content at predicted optimized protein conditions were found to be 21.23% (w/w), which shows an increment of 35.23% compared to before optimization. Yeast growth and sugar content in yeast cell were previously reported to be related to the types of sugar used as substrate (Liong *et. al.*, 2002) and growth of *S. cerevisiae* in beet molasses increased with the increment of total sugar consumed (El-Diwany *et.al.*, 1992).

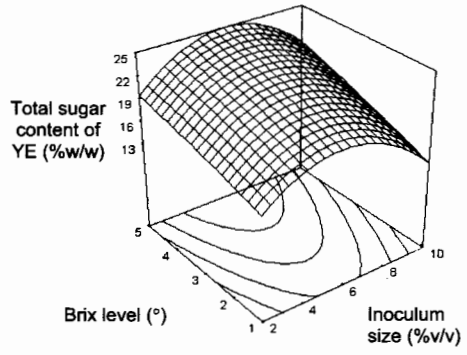

Figure 5: Response surface for dependent variable, total sugar content of YE

Cell growth was also found to increase in tandem with the increment of total sugar in yeast cell. Results obtained suggested that total sugar in medium exceeded the need for cell growth and was used as intracell conservatory source (Kratochvilova, 1990). This also suggested that substrate sugar inhibition may not have happened within the experimental Brix region (1-5°).

Evaluation of YE vitamin-B content

Vitamin-B content yeast cell was measured in terms of total single vitamin-B consisting of B_3 (niacin), B_6 (pyridoxine), B_2 (riboflavin) and B_1 (thiamin). Vitamin-B content varied within the range 43.85-117.25 mg/100 g sample. ANOVA results (Table 7) indicates good data fitting for the second-order model used and LOF was insignificant. Both independent variables of inoculum size and substrate Brix level showed significant influence towards vitamin-B content of yeast extract, with the earlier producing greater effect.

Table 7: Analysis of variance for dependent vitamin-B variable content of YE (Y_4)

Source	Sum of squares	DF	Mean square	F-value	Probability>F
Regression:					
Linear	2320.18	2	1160.09	2.33	0.1477
Quadratic	4524.84	3	1508.28	23.24	0.0005[a]
Cubic	28.20	2	14.10	0.17	0.8520
Residual	426.05	5	85.12	-	-
Model:	6845.02	5	1369	21.10	0.0004[a]
Lack-of-Fit	160.13	3	53.38	0.73	0.5874
Pure error	294.12	4	73.53	-	-
Factor:	**Coefficient estimate**	**DF**	**Standard error**	**t for H_o coefficient=0**	**Probability y>t**
Inoculum size (% v/v)	-19.34	1	3.29	-5.88	0.0006[a]
Brix level (°)	-3.54	1	3.29	-1.08	0.3174
R^2= 0.9378					

Figure 6 shows the predicted dependence of vitamin-B content on inoculum size and substrate Brix level, based on the equation in Table 4. Response surface shows that lower inoculum size produced higher vitamin-B content for all Brix level, and a maximum response can be obtained. Highest vitamin-B value was predicted to be 109.62 mg/100g sample at inoculum size 4.83% (v/v) and Brix level 2.08°. This marked an increase of 17.5% as compared to initial value obtained at inoculum size 2% (v/v) and Brix level 1°. Vitamin-B content was predicted to be 89.73 mg/100g sample at optimized protein conditions and this marks a decrease of 3.8% as compared to before optimization conditions. Total vitamin-B content obtained was found to be high and this agrees with previous studies that showed better vitamin-B accumulation by yeast cultivated on natural undefined medium. Natural media were found to be rich in inorganic elements and growth substances that fortify vitamin-B synthesis, supply essential growth vitamins and act as vitamin precursors (Kratochvilova, 1990).

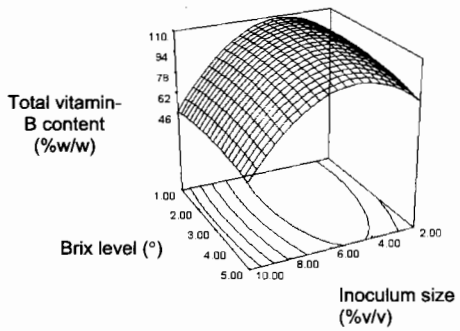

Figure 6: Response surface for dependent variable, total vitamin-B content of YE

Evaluation of YE 5'-ribonucleotide content

5'-ribonucleotide content was obtained from the total of 5'-inosine-monophosphate (IMP) and 5'-guonosine-monophosphate (GMP), which were reported to contribute to umami taste in yeast extract products (Matheis, 1999). Table 8 shows the ANOVA results for the model obtained that explain the response of the dependent variable, Y_5, 5'-ribonucleotide content of yeast extract. Data fitting was satisfactory by the second-order model used and lack-of-fit was not significant. Inoculum size showed greater significance towards 5'-ribonucleotide content compared to Brix level (Table 4).

Table 8: Analysis of Variance for Dependent Variable 5'-ribonucleotide Content of YE (Y_5)

Source	Sum of squares	DF	Mean square	F-value	Probability>F
Regression:					
Linear	28.08	2	14.04	3.85	0.0576
Quadratic	34.48	3	11.49	40.28	<0.0001[a]
Cubic	0.43	2	0.22	0.69	0.5431
Residual	1.56	5	0.31	-	-
Model:	62.56	5	12.51	43.86	<0.0001[a]
Lack-of-Fit	1.60	3	0.53	5.31	0.0702
Pure error	0.40	4	0.10	-	-

Factor:	Coefficient estimate	DF	Standard error	t for H_o coefficient =0	Probability>t
Inoculum size (% v/v)	2.02	1	0.22	9.27	<0.0001[a]
Brix level (°)	0.77	1	0.22	3.53	0.0097[a]
R^2= 0.9691					

Response surface (Figure 7) shows that at higher Brix level, increment of inoculum size increased 5'-ribonucleotide content greatly. At higher inoculum size, the increment of Brix level also increased 5'-ribonucleotide content greatly. This shows that 5'-ribonucleotide content in yeast cell may be further increased with the increment of both inoculum size and substrate Brix level. Highest 5'-ribonucleotide content was predicted to be 20.22 mg g^{-1} sample, which was obtained from inoculum size 10.0% (v/v) and Brix level 5°. This marked an increment of 38.0% compared to initial value obtained before protein optimization. 5'-ribonucleotide content at optimized protein conditions was predicted to be 18.21 mg g^{-1} sample, which showed an increment of 24.3%. However, it was lower than the reported 3.67% (w/w) of total GMP and IMP from enzymatically autolyzed *S. cerevisiae* (Chae *et.al.*, 2001), and commercial YE with IMP and GMP ranging from 1.5-6.0% (w/w) (Nagodawithana, 1992). This may suggest that enzymatically autolyzed yeast cells produce better 5'-ribonucleotides content compared to mechanically ruptured cells. With IMP and GMP playing major roles in producing umami effects, 5'-ribonucleotide content from pineapple waste medium was higher than some food products. Average IMP and GMP content in meat products were 0.26% (w/w) and 0.003% (w/w) respectively, while in fish products were 0.66% w/w and 0.01% (w/w) respectively (Matheis, 1999).

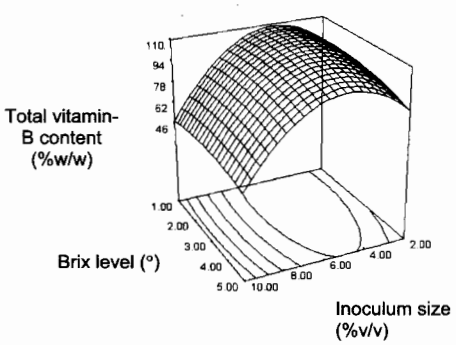

Figure 7: Response surface for dependent variable, total 5'-ribonucleotides content of YE

A confirmation experiment that was performed at optimized protein conditions (inoculum 7.8% v/v, Brix 3.0°) produced protein content of 66.49 % (w/w), growth 15.70 g l^{-1}, vitamin-B content of 90.6 mg per 100g sample, 5'-ribonucleotide content of 17.8 mg g^{-1} sample and total sugar content of 25.06% (w/w). Results obtained are close to the predicted values, indicating the reliability of the model used.

CONCLUSION

Pineapple waste is an economical fermentation medium for SCP production with growth 15.70 g l^{-1}, yeast cell protein content of 66.49% (w/w), vitamin-B content of 90.6 mg/100g sample, 5'-ribonucleotide content of 17.8 mg g^{-1} sample and total sugar content of 25.06% (w/w). RSM application succeeded in optimizing protein content with proven reliability of the model used.

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