Statistical design and optimization of nutritional value production by an oleaginous yeast *Yarrowia lipolytica* cultured in industrial – waste molasses

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Abstract. Abundant by-products from sugar mills as industrial-waste molasses can be used as a carbon source in yeast culture media. *Yarrowia lipolytica* is an interesting yeast used as a candidate for cultivation in molasses medium. Here, we used response surface methodology to derive a statistical model for the individual and interactive effects of pH, temperature, and shaking speeds on the production of yeast cells. Cultivation conditions of yeast were optimized using Design Expert based on a 2^3 factorial central composite design (CCD) for maximum yeast cell production. Optimal conditions for maximum *Y. lipolytica* 5151 cell masses were as follows: pH, 6.45; temperature, 30ºC; Shaking speed, 165 rpm. The Design Expert represented the maximal numerical solution with a predicted cell mass production level at 8.96 g/L. The experimental production of *Y. lipolytica* 5151 cell mass yielded 8.27 g/L that is 7.67% deviated from the model. Whereas, the model of TISTR 5621 was not adequate for prediction. Yeasts cultured under statistic prediction provide 55.94% and 51.25% of total protein. Amino acid content and vitamin B1 (1.06 mg and 1.47 mg per 100 g of dried *Y. lipolytica* 5151 and 5621, respectively) provided the relevant information for an alternative supplement in aquatic feed.

Keyword. Molasses; An oleaginous yeast; statistic design

1 Introduction

Yarrowia lipolytica is classified as an oleaginous yeast that has a high ability to produce lipids or triacylglycerides (TAGs) depended on a culture condition. It becomes to be an attractive alternative source to decrease the dependency on vegetable and mineral oil. Moreover, it was used as a non-pathogenic microorganism for lipid production applied as components in coatings, paints, personal care products, production of fine chemicals, and biodiesel [1]. *Y. lipolytica* can produce and accumulate 30-43% lipid in the cell, and it also can produce and secret a high level of protein [2, 3].

A study in a yeast species known as *saccharomyces cerevisiae* as a nutritional yeast containing protein, vitamins, minerals, and antioxidants. However, the composition and nutritive value of *Y. lipolytica* biomass are still limited. Due to the ability to secrete protein at a high level depending on the carbon source in contrast to *S. cerevisiae*, *Y. lipolytica* has become the most interesting organism for many applications in producing and secretion of several organic acids and fragrances. [4, 5]

The development of an economic culture medium for *Y. lipolytica* needs to obtain a high quantity of its biomass. The satisfied medium must compose of the basic requirements for cell biomass. The carbon substrate is a main role in biosynthesis and energy generation [6]. There are various carbon sources in microbial culture. Since *Y. lipolytica* can use unusual carbon sources, such as hydrocarbons, several studies focused on increasing yeast biomass by using different carbon sources. Juszczyk et al. [7] used waste derived from the production of ethyl esters of polyunsaturated fatty acids of flaxseed oil to produce biomass of *Y. lipolytica*. Wrobel-Kwiatkowska et al. [8] developed *Y. lipolytica* media containing different kinds of honey for product improvement of Kynurenic acid. Media development for *Y. lipolytica* growth is relevant because media cost is a significant problem of the large-scale yeast cultivation for industrial applications. Therefore, Molasses is a by-product of sugar processing, and it also can be an efficient, low-cost carbon source in a *Y. lipolytica* culture.

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The response surface methodology (RSM) is a mathematical and statistical technique to estimate the experimental variability, confirm the adequacy between the proposed model and the experimental data, predict the observed response and construct the results in a 3D empirical model [9]. Due to the tedious task of the classical "one-factor-at-a-time" method in investigating interactions between variables, RSM method has been applied in biological research, food, and feed technology applications. This study aims to optimize the best conditions for *Y. lipolytica* TISTR 5151 and 5621 to grow in industrial-waste molasses using the Design expert program. *Y. lipolytica* could be an alternative source or ingredient for fish and shrimp feeds.

2 Materials and Methods

2.1 Materials

Y. lipolytica TISTR 5151 and 5621 were purchased from Thailand Institute of Scientific and Technological Research (TISTR), Thailand. Peptone, Yeast extract, Malt extract, Agar, Hydrochloric acid, Sodium hydroxide used for cell culture and analysis were of analytical grade. They were purchased from Sigma, USA, and HiMedia, India. Molasses was supported by a local sugar factory in Suphanburi province, Thailand. Design Expert ver. 10.0.2 was used in the optimization analysis of the yeast cell growth.

2.2 Yeast cells preparations

Y. lipolytica TISTR 5151 and 5621 were grown on modified media composed of 5.6% molasses as a carbon source, 0.5% peptone, 0.3% yeast extract, and 0.3% malt extract. The yeasts were cultured in three various optimized factors (pH, temperature, and shaking speed). Moisture contents from a moisture analyzer were relative to yeast cell dry weight values, as the following equation shows below [10].

$$\%\ \text{Moisture content} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight of sample (g)}}$$

2.3 Experimental Design setup for yeast cell growth optimization

In the research, three selected factors were pH, temperature, and shaking speed. These factors were used to determine their effects on two strains of yeast cell growth in the modified culture medium containing molasses as a carbon source. The experiments used a central composite design. Table 1 shows the factors and their levels where the high level indicates the highest range of the factors, and the low level reveals the lowest of the factors. Experiment design Tables 2 and 3 of *Y. lipolytica* TISTR 5151 and 5621 were constructed using the Design Expert Software v.10.0.2, and the experiment data was analyzed using the same software.

2.4 Nutritional value analysis of dry yeasts.

Free amino acid contents and vitamin B1 were determined from each yeast cells. HPLC technique was applied to the quantification of each amino acid and vitamin B1 of 100g of each dry yeast [11, 12].

Table 1. The levels of selected factors

Factors	Low level	High level
pH	5	9
Temperature (Celsius)	28	32
Shaking speed (rpm)	150	250

Table 2. Experimental setup of *Y. lipolytica* TISTR 5151 that has been constructed by using 2^3 factorial analyses by Design Expert software v.10.0.2

Run	Factor1 A: pH	Factor2 B: temperature (Celsius)	Factor3 C: Shaking speed (rpm)
1	7	30	200
2	7	30	200
3	7	28	200
4	7	30	200
5	9	28	250
6	9	32	250
7	7	30	150
8	5	30	200
9	5	28	150
10	9	28	150
11	7	30	200
12	9	30	200
13	5	32	250
14	7	30	200
15	7	30	200
16	7	30	250
17	7	32	200
18	5	32	150
19	5	28	250
20	9	32	150

Table 3. Experimental setup of *Y. lipolytica* TISTR 5621 that has been constructed by using 2^3 factorial analyses by Design Expert software v.10.0.2

Run	Factor1 A: pH	Factor2 B: temperature (Celsius)	Factor3 C: Shaking speed (rpm)
1	7	30	200
2	7	30	200
3	7	32	200
4	5	32	150
5	7	30	200
6	9	28	250
7	7	30	150
8	9	30	200
9	7	28	200
10	7	30	200
11	9	32	150
12	7	30	200
13	5	32	250
14	7	30	250
15	5	28	250
16	5	30	200
17	5	28	150
18	9	32	250
19	7	30	200
20	9	28	150

3 Results and discussions

3.1 Screening of Factors Affecting on *Y. lipolytica* TISTR 5151 and 5621 Growth

pH, temperature, and shaking speed were applied to study the practical effect on yeast growth. 2^3 level factorial method in Design Expert was used to optimize the condition to obtain the highest biomass yields. Tables 4 and 5 show 20 runs of experiments that reveal obtained yeast cell dry weight results. The response was analyzed using the ANOVA component of central composite design (CCD) found in response surface methodology of the Design-Expert software v10.0.2 at 95% confidence level. For *Y. lipolytica* TISTR 5151, the highest yield of biomass was obtained at pH7, 30°C, and 200 rpm of shaking speed, whereas the condition for the highest yield of biomass of *Y. lipolytica* TISTR 5621 at pH5, 32°C, and 150 rpm of shaking speed. The design matrix for CCD experiments and the experiment results and the predicted responses for Cell Dry Weight (CDW) of Y. lipolytica TISTR 5151 and 5621 were regressed using a quadratic polynomial equation and linear equation for TISTR 5151 and 5621, respectively. The two regression equations were as follows:

CDW of *Y.lipolytica* TISTR 5151 =
-23.12384 - 0.22690A +1.64501B -0.00191783C+ 0.010257AB - 0.000289708AC+ (0.000239625BC-0.00205265A^2-0.028965B^2-0.00000947090C^2

$$\text{CDW of } Y.\,lipolytica \text{ TISTR } 5621 = 0.75398 - 0.00282833A + 0.00377B - 0.0003456C$$

where A is pH, B is the temperature (Celsius), and C is shaking speed (rpm)

Table 4. Experimental results of *Y. lipolytica* TISTR 5151 cell dry weight on different factors using Design Expert software v.10.0.2 based on 2^3 level factorial CCD

Run	Factor1 A: pH	Factor2 B: Temperature (Celsius)	Factor3 C= Shaking speed (rpm)	Response Cell Dry Weight (CDW) (10 g/L)
1	7	30	200	0.891
2	7	30	200	0.891
3	7	28	200	0.721
4	7	30	200	0.891
5	9	28	250	0.504
6	9	32	250	0.828
7	7	30	150	0.876
8	5	30	200	0.877
9	5	28	150	0.733
10	9	28	150	0.764
11	7	30	200	0.896
12	9	30	200	0.895
13	5	32	250	0.748
14	7	30	200	0.891
15	7	30	200	0.891
16	7	30	250	0.865
17	7	32	200	0.837
18	5	32	150	0.797
19	5	28	250	0.767
20	9	32	150	0.814

Table 5. Experimental results of *Y. lipolytica* TISTR 5621 cell dry weight on different factors using Design Expert software v.10.0.2 based on 2^3 level factorial CCD.

Run	Factor1 A:pH	Factor2 B:Temperature (Celsius)	Factor3 C= Shaking speed (rpm)	Response Cell Dry Weight (CDW) (10 g/L)
1	7	30	200	0.765
2	7	30	200	0.765
3	7	32	200	0.746
4	5	32	150	0.845
5	7	30	200	0.765
6	9	28	250	0.695
7	7	30	150	0.771
8	9	30	200	0.784
9	7	28	200	0.824
10	7	30	200	0.765
11	9	32	150	0.842
12	7	30	200	0.765
13	5	32	250	0.784
14	7	30	250	0.813
15	5	28	250	0.799
16	5	30	200	0.732
17	5	28	150	0.787
18	9	32	250	0.766
19	7	30	200	0.764
20	9	28	150	0.794

3.2 Analysis of Variance (ANOVA) for yeast cell dry weight

The analysis of variance (ANOVA) for the CCD experiment was performed to estimate the coefficient of the model and determine the significance of each parameter (Table 6). The F-values and P-values were used to identify the effect of each factor on obtained cell dry weight in each strain of *Y. lipolytica.* The model of *Y. lipolytica* TISTR 5151 exhibited correlation with the experimental data with high F-value and P-value < 0.05, which implied the significance of the model. In contrast, low F-value and P > 0.05 of *Y. lipolytica* TISTR 5621 model indicated that this model was not significant. By inspecting each F-values and P-values of the Y. lipolytica TISTR 5151 model, it can be seen that the effect on biomass of Y. lipolytica cultured in media containing molasses carbon source depended on the temperature. Different strains in *Y. lipotica* had various physiological and metabolism properties and required other conditions to grow.

The previous studies were found that high temperature affects sugar alcohol production [13]. In TISTR 5151, R^2 value (0.90) indicated that the model was reliable for predicting biomass. The lack of fit F-value which was 1.87 (P-value 0.1577 < 0.05), also replied that the model was adequate for prediction.

Three-dimensional response surface plots were constructed to show the effect of pH, temperature and shaking speed on cell dry weight of *Y. lipolytica* TISTR 5151 (Figure 1). The impact of individual variables is paired within the experiment range. This graphical visualization allows investigating the relationships between the experimental levels of each factor and the response. There was a large interactive effect of

temperature and another factor on cell dry weight. At moderate temperature, the strongest yeast biomass increases *Y. lipolytica* TISTR 5151 cultured in the media containing molasses as a carbon source. When dry cell weight was considered, the interactions between pH and temperature and the interaction between shaking speed and temperature are strong (Figure 1A and 1C). In contrast, it is not been shown in the interaction between shaking speed and pH (Figure 1C).

The optimal levels of those factors can be deduced from a 3D response and the equation obtained from regression analysis. The model predicted that the maximum cell dry weight (8.96 g/mL) was located at A= 6.45, B = 30 Celsius, and C= 165 rpm. The experiments were performed in the predicted cell culture condition to examine the validity of this model. The average obtained biomass was 8.27 g/L. It was close to the predicted values and supported the suitability of the model in this study.

Table 6. Analysis of variance (ANOVA) for the experimental results of the CCD

Source	*Y. lipolytica* TISTR 5151					*Y. lipolytica* TISTR 5621				
	SS	df	SM	F-value	P-value	SS	df	SM	F-value	P-value
Model	0.16	9	0.017	9.43	0.0008	0.0039	3	0.0013	1.09	0.3815
A	0.0042	1	0.0043	2.33	0.1577	0.00032	1	0.00032	0.27	0.6103
B	0.033	1	0.033	17.80	0.0018	0.00057	1	0.00057	0.48	0.4983
C	0.00017	1	0.00017	0.0094	0.7650	0.00298	1	0.00298	2.52	0.1319
AB	0.013	1	0.013	7.33	0.0221	-	-	-	-	
AC	0.0067	1	0.0067	3.65	0.0850	-	-	-	-	-
BC	0.0046	1	0.0046	2.50	0.1450	-	-	-	-	-
A^2	0.00019	1	0.00019	0.10	0.7573	-	-	-	-	-
B^2	0.037	1	0.037	20.08	0.0012	-	-	-	-	-
C^2	0.0015	1	0.0015	0.84	0.3813	-	-	-	-	-
Lack of fit	0.018	0.0037	0.0037	1.87	0.1577	0.019	11	0.0017	2.86	0.003
R^2=0.90						R^2= 0.16				

A, pH; B, temperature; C, shaking speed
*P < 0.05 are significant
SS: Sum of squares; df: degree of freedom; SM: mean squares

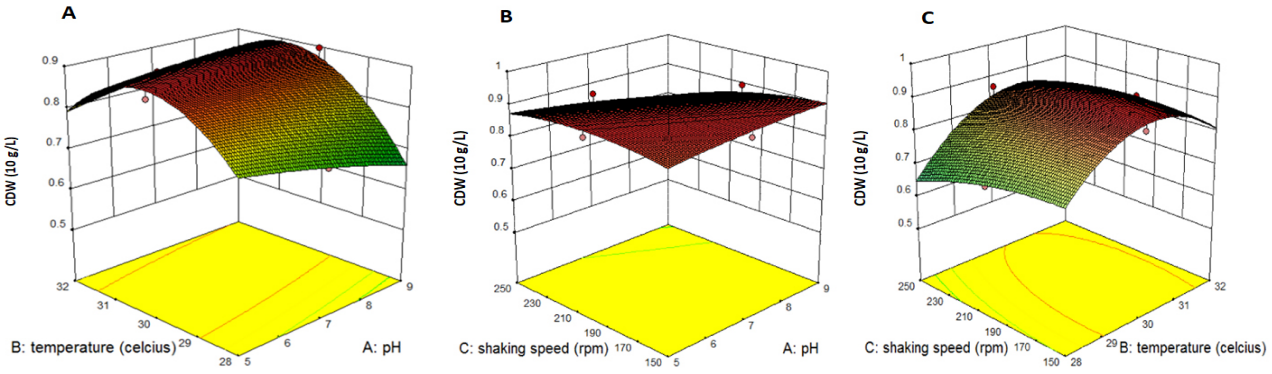

Figure 1. Response surfaces (A) A 3D Graphical combination between pH and temperature to the cell dry weight of *Y.lipolytica* TISTR 5151 (B) A 3D Graphical combination between pH and shaking speed to the cell dry weight of *Y.lipolytica* TISTR 5151 (C) A 3D Graphical combination between temperature and shaking speed to the cell dry weight of *Y.lipolytica* TISTR 5151.

Table 7. Validation of the optimization values predicted by RSM and the experiment

Factor A pH	Factor B Temperature (Celsius)	Factor C Shaking speed (rpm)	Predicted Cell dry weight (g/mL)	Cell dry weight from the experiment (g/mL)		
				Run 1	Run 2	Run 3
6.45	30	165	8.96	8.84	8.09	7.89
				Average: 8.27, Error: 7.67%		

Table 8. Percent composition of yeast biomass and yeast protein concentrates

Components*	*Y. lipolytica* TISTR 5151	*Y. lipolytica* TISTR 5621	Method
Total protein (%)	55.94	51.25	AOAC 2010.AOAC official method 984.13
Total lipid (%)	0.73	1.54	AOAC 2010.AOAC official method 2003.05
Ash (%)	7.43	6.47	AOAC 2010.AOAC official method 942.05
Total fiber (%)	0.61	0.58	AOAC 2010.AOAC official method 962.09

* Percentages on a dry basis

3.3 Yeast composition, amino acid, and vitamin B1 content

The biomass compositions are evaluated for two *Y. lipolytica* different strains, TISTR 5151 and 5621. Protein concentrations of whole yeast biomass TISTR 5151 and TISTR 5621 cultured in the predicted condition were 55.94 % and 51.25%, respectively (Table 8). The amino acid profiles of yeast biomass in both strains are shown in Table 9. The results of amino acid content in dry yeast cells in both yeast strains were not significantly different. The high content of Lysine is found in yeast biomass (Table 9). This amino acid is essential dietary protein for the growth of catla (*Catla catla*), the major South Asian carp [14], and Nile tilapia [15, 16]. Aquatic feeds for farmed fish and shrimp need each amino acid to depend on marine species [17]. Using *Y. lipolytica* dry cell in feed formulation can be an alternative approach to increase essential amino acids for aquatic feeds. Moreover, Yeast cells also contained vitamin B1 (Thiamin) as another supplement for fish feed (Table 10) [18].

Table 9. Amino acid composition analysis for the yeast biomass

Amino acids (mg/100 g protein)	***Y. lipolytica* TISTR 5151**	***Y. lipolytica* TISTR 5621**
Alanine	6,140	4,512
Arginine	2,481	2,532
Aspartic acid	3,782	4,514
Cystine	1,515	1,546
Glutamic acid	6,785	5,992
Glycine	6,765	4,482
Histidine	889	1,054
Isoleucine	1,456	1,861
Leucine	2,396	2,573
Lysine	5,347	4,921
Methionine	717	782
Phenylalanine	1,367	1,646
Proline	2,501	2,431
Serine	2,631	2,189
Threonine	1,813	2,201
Tryptophan	971	714
Tyrosine	1,132	1,221
Valine	1,689	2,264

Table 10. Vitamin B1 analysis for the yeast biomass

Vitamin B1 (mg/100 g protein)	***Y. lipolytica* TISTR 5151**	***Y. lipolytica* TISTR 5621**
	1.06	1.47

4 Conclusion

Different *Y. lipolytica* isolates derived from Thailand Institute of Scientific and Technological Research might require various factors for maximizing yeast biomass. RSM is a powerful method for program optimization and identification of the relative significance of different factors, interactions between factors, and optimal level of test variable. The method has been successfully applied to optimize critical medium components for mycelium biomass of *Lentinus squarrosulus* [19], obtain the optimum temperature and drying time in making cocktail yeast molds [20], and maximize biomass of *Purpureocillium lilacinum* KU8 [21]. *Y. lipolytica,* a non-pathogenic organism, is considered as a model for a study of protein secretion, hydrophobic substrate utilization, and peroxisome biogenesis, dimorphism and mitochondrail complexI [22]. Protein, amino acid content, and vitamin B1 were found in *Y. lipolytica* dry cell, indicating that the dry cell yeast might be applied as a significant protein-rich ingredient in aquafeeds. Nutritional quality of *Saccharomyces cerevisiae*, *Cyberlindnera jadinii*, *Kluyveromyces marxianus*, *Blastobotrys adeninivorans,* and *Wicker-hamomyces anomalus* had been studied to use them as a replacement for fishmeal and soy protein in the diets of Atlantic salmon [23].

This study has been successfully optimized critical growth conditions in using molasses as a carbon source for *Y. lipolytica* varieties cultivated in Thailand. This alternative medium could reduce media cost that a significant issue for large-scale yeast cultivation.

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References

1. A. Beopoulos, J. Verbeke, F. Bordes, M. Guicherd, M. Bressy, A. Marty, J.M. Nicaud, Metabolic engineering for ricinoleic acid production in the oleaginous yeast Yarrowia lipolytica, Applied Microbiology and Biotechnology, **98**, 1 (2014): 251-62
2. S. Papanikolaou, G. Aggelis, Lipid production by Yarrowia lipolytica growing on industrial glycerol in a single-stage continuous culture, Bioresource Technology, **82**, 1 (2002): 43-9
3. J.-M. Nicaud, Yarrowia lipolytica, Yeast, **29**, 10 (2012): 409-418
4. P. Saçakl1, B.H. Koksal, A. Ergun, B. Özsoy, Usage of brewer's yeast (Saccharomyces cerevisiae) as a replacement of vitamin and trace mineral premix in broiler diets, Revue de Médecine Vétérinaire, **164**, 1 (2013): 39-44
5. M.T. Pacheco, G.M. Caballero-Córdoba, V.C. Sgarbieri, Composition and nutritive value of yeast biomass and yeast protein concentrates, Journal of Nutritional Science and Vitaminology (Tokyo), **43**, 6 (1997): 601-12
6. G.M. Walker, Fermentation (Industrial) : media for industrial fermentations, (E.o.f.m. editor (Ed.) Carl A. Batt. 2nd. ed. London : Academic Press,2014): 769-777
7. P. Juszczyk, W. Rymowicz, A. Kita, A. Rywińska, Biomass production by Yarrowia lipolytica yeast using waste derived from the production of ethyl esters of polyunsaturated fatty acids of flaxseed oil, Industrial Crops and Products, **138** (2019): 111590
8. M. Wróbel-Kwiatkowska, W. Turski, P. Juszczyk, A. Kita, W. Rymowicz, Improved Production of Kynurenic Acid by Yarrowia lipolytica in Media Containing Different Honeys, Sustainability, **12**, 22 (2020): 9424
9. L.A. Sarabia, M.C. Ortiz, 1.12 - *Response Surface Methodology* (S.D. Brown, R. Tauler, B. Walczak (Eds.), Comprehensive Chemometrics, Elsevier, Oxford, (2009): 345-390
10. E. Li, R. Mira de Orduña Heidinger, A rapid method for the determination of microbial biomass by dry weight using a moisture analyser with an infrared heating source and an analytical balance, Letters in applied microbiology, **50** (2010): 283-8
11. P. Herbert, P. Barros, N. Ratola, A. Alves, HPLC Determination of Amino Acids in Musts and Port Wine Using OPA/FMOC Derivatives, Journal of Food Science, **65**, 7 (2000): 1130-1133
12. F. Batifoulier, M.A. Verny, E. Chanliaud, C. Rémésy, C. Demigné, Effect of different breadmaking methods on thiamine, riboflavin and pyridoxine contents of wheat bread, Journal of Cereal Science, **42**, 1 (2005): 101-108
13. S. Hackenschmidt, F. Bracharz, R. Daniel, A. Thürmer, S. Bruder, J. Kabisch, Effects of a high-cultivation temperature on the physiology of three different Yarrowia lipolytica strains, FEMS Yeast Research, **19**, 7 (2019): 31605534
14. J. Ravi, K.V. Devaraj, Quantitative essential amino acid requirements for growth of catla, Catla catla (Hamilton), Aquaculture, **96**, 3 (1991): 281-291
15. C.B. Santiago, R.T. Lovell, Amino Acid Requirements for Growth of Nile Tilapia, The Journal of Nutrition, **118,** 12 (1988): 1540-1546
16. R.d.S. Romaneli, do Nascimento, T. Matias Torres, Gous, R. Mervyn, Reis, M. de Paula, Mansano, C. Fernando Menegasso, Khan, K. Ullah, Sakomura, N. Kazue, & Fernandes, J. Batista Kochenborger., Response of Nile tilapia (Oreochromis niloticus) to lysine: Performance, body composition, maintenance and efficiency of utilization, Aquaculture, **538** (2021): 736522
17. A.J.P. Nunes, M.V.C. Sá, C.L. Browdy, M. Vazquez-Anon, Practical supplementation of shrimp and fish feeds with crystalline amino acids, Aquaculture, **431** (2014): 20-27
18. F.G. Laura Gasco, G. Maricchiolo, L. Genovese, S. Ragonese, T. Bottari, G. Caruso, *Supplementation of Vitamins, Minerals, Enzymes and Antioxidants* (Feeds for the Aquaculture Sector. SpringerBriefs in Molecular Science. Springer, Cham, 2018): 63-103
19. R. Ahmad, N.K.N. Al-Shorgani, A.A. Hamid, W.M.W. Yusoff, F. Daud, Optimization of medium components using response surface methodology (RSM) for mycelium biomass and exopolysaccharide production by *Lentinus squarrosulus*, Advances in Bioscience and Biotechnology, **4**, 12 (2013): 1079-1085
20. R. Rahmawati, Hunaefi, D., Basriman, I., Saputra, D., Apriliani, A.A. and Jenie, B.S.L., Optimization of temperature and drying time of indigenous cocktail yeast mold culture using response surface methodology (RSM), Food Research, **4**, 2 (2020): 389 - 395
21. M. Mousumi Das, M. Haridas, A. Sabu, Process development for the enhanced production of bio-nematicide *Purpureocillium lilacinum* KU8 under solid-state fermentation, Bioresource Technology, **308** (2020): 123328
22. J.M. Nicaud, Yarrowia lipolytica, Yeast, **29**, 10 (2012): 409-418
23. J.O. Agboola, M. Øverland, A. Skrede, J.Ø. Hansen, Yeast as major protein-rich ingredient in aquafeeds: a review of the implications for aquaculture production, Reviews in Aquaculture, **13**, 2 (2021): 949-970