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Bioconversion and Deodorization of Shrimp Processing Waste by *Xerocomus badius* and Inhibitory Activity of Converted Product on Angiotensin I-converting Enzyme

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Abstract: Shrimp processing waste were converted by *Xerocomus badius*, bioconversion conditions and then optimized through Plackett-Burman design, path of steepest ascent and response surface methodology in order to recycle the solid waste efficiently. Based on the results of Plackett-Burman design and path of steepest ascent, a central composite design was applied for the regression models of the highest mycelium biomass and ACE inhibitory rate of water extract from the mycelium. The optimal cultural conditions for the highest mycelium biomass and ACE inhibitory rate were determined to be: A solid to liquid ratio of 12.421, 7.964% of content of bran powder and 1.117% of glacial acetic acid. Fermenting with the optimal cultural conditions resulted in an mycelium biomass of 28.890 g L^{-1} and ACE inhibitory rate of water extract (0.400 g mL^{-1}) of 69.746% (IC_{50} = 0.212 mg mL^{-1}). These values are consistent with the values predicted by the corresponding regression models (RSD<5%) and improved by 38.210 and 38.948%, respectively compared to that gained before optimization. The yield of ACE inhibitory water extract reached to 13.926 g L^{-1} and was improved by 44.596%. Besides, bioconversio, *Xerocomus badius* has effective deodorization effect. This study improved that it is practicable and efficient to recycle the shrimp processing waste with bioconversion by *Xerocomus badius* and afforded an experimental and theoretical foundation for low-cost reusing shrimp processing waste and producing ACE inhibitor in industrial scale.

Key words: Shrimp processing waste, bioconversion, *Xerocomus badius*, ACE inhibitor, deodorization

INTRODUCTION

Shrimp is a popular type of seafood and its production is a major agricultural product in coastal areas. In last two decades, production of shrimp has increased year by year. So shrimp processing industry has developed rapidly. During the processing, the head, shell, tail portion and small fry with low edible value are removed and discarded as waste which makes up approximately 80% weight of the raw materials (Balogun and Akegbejo-Samsons, 1992). Uncontrolled dumping and hauling of Shrimp Processing Waste (SPW) has caused a wasting of natural resources and eutrophication of the coastal and marine environment (Islam *et al*., 2004), because it contain large amounts of nutritive components, mineral calcium and phosphorus (Heu *et al*., 2003). The problem would become increasingly severe without effective measures. Therefore, large-scale recycling of SPW has been paid attention increasingly.

SPW has been reused as fertilizer and feedstuff (Carr *et al*., 1992; Watkins *et al*., 1982; Evers and Carroll, 1996; Fagbenro and Bello-Olusoji, 1997) previously. In recent years, the solid waste has been treated with chemical methods for nutrients comprise and bioactive substances, such as protein, carotenoids, chitin, chitosan and astaxanthin were extracted from the waste resource (Synowiecki and Al-Khateeb, 2000; Eswaralakshmi and Saravanan, 2012; Sachindra *et al*., 2006; Percot *et al*., 2003; Manni *et al*., 2010; Sanchez-Camargo *et al*., 2011). However, the above-mentioned methods can only reuse a small part of the waste resource. Furthermore, some of them require large quantities of strong acid or strong base (Abdou *et al*., 2008) which may cause secondary pollution of environment and increase the cost. Moreover, most products of them have low added value. Additionally, SPW smells fishy, this always limits its reclamation. However, there has not been any report about the technology of deodorization of SPW.

With the development of modern biotechnology, microorganisms has increasingly been used in waste treatment. Bioconversion of solid wastes remains a vital part of the overall biological methods for treatment of solid wastes. The SPW has also been treated with biological methods, such as being fermented by lactic acid

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bacteria (Phuvasate and Su, 2010), *Bacillus cereus* and *Exiguobacterium acetylicum* (Rao and Stevens, 2005) to recover chitosan and chitin and being salt-fermented for sauce with tenderized effect on meat and liquor with antioxidant ability (Kim *et al*., 2005).

Edible/medicinal mushrooms are traditionally cultured on lignocellulosic waste for their fruiting bodies due to their nutritional, healthy and drug effective value. Attention has more and more been paid to Angiotensin I-Converting Enzyme (ACE) inhibitors which has potential of antihypertensive drugs, from edible/medicinal mushrooms. The process of bioconversion from solid wastes to bioactive substances needs no chemicals, so it is environmentally friendly. It can also reduce costs and enhance the added value of its products. Meanwhile, the SPW was deodorized after being converted in the present study which has important value in treatment of aquatic byproducts. However, there has not been any report about using any fungal species for reclamation of SPW.

Therefore, SPW was converted by *Xerocomus badius* originally in the present study. Conditions of the bioconversion with *X. badius* were optimized for enhancing the efficiency of the bioconversion. Moreover, the deodorization effect of the bioconversion, ACE inhibitory activity of water extract from the mycelium produced by the conversion was investigated. The aim of this study was to provide a theoretical and practical basis for large-scale economical production of natural ACE inhibitor from liquid fermented *X. badius* and establish a novel method to recycle SPW by converting with *X. badius*.

MATERIALS AND METHODS

Pretreatment of SPW: SPW (purchased from a local shrimp processing plant) were dried at 50°C and pulverized into powder.

Preliminary bioconversion: *Xerocomus badius* which were maintained on pitched potato dextrose agar (PDA, composed of 200 g L^{-1} potato, 20 g L^{-1} glucose, 20 g L^{-1} agar powder) medium at 4°C in the laboratory, were activated in petri dishes on the solid medium (PDA) at 25°C. The stain was cultivated in 250 mL shake-flasks containing 100 mL of liquid medium (was same as the solid medium except of non-agar) on a shaking incubator at 120 rpm and 25°C for 3 days which served as control and liquid seeds for the bioconversion. The liquid medium for initial bioconversion composed of 100 mL deionized water (containing 1.3% of acetic acid) and 6.667 g solid compounds (containing 94% of SPW and 6% of bran powder). The liquid fermentations of bioconversion were carried out in 250 mL shaken flasks containing 100 mL cultural medium, as well as 8 mL of liquid seeds at 25°C and 120 rpm. Liquid medium without any fungal species inoculated was treated in the same conditions for control. Measurement of deodorization effect: After fermentation, the fishy smell of bioconverted products and the blank SPW medium for control was evaluated through questionnaire investigation: there are 12 subjects (evenly divided between male and female, aged 18-25) were asked to smell each sample and then choose 1 option from the total 5 (extremely heavy, comparable heavy, a little, slight, no fishy smell which were denoted as 0-4 points). The blank liquid medium served as control. The better the deodorization effect, the higher the score.

Preparation of water extracts: After the deodorization effect being evaluated, all samples, including the bio-converted products, the control SPW medium and the fermented products of *X. badius* cultured in PDA were filtered through two layers of gauze and divided into solid constituent and broth. Then, fermented mycelium of *X. badius* and the solid constituent from the control SPW medium were flushed with distilled water to colorless, lyophilized (with a lyophilizer, FD-2B, Henan Brothers Equipment Co., Ltd., China) to constant weight and weighed. These dried samples were pulverized, added to distilled water (1:50, w/v) and shaked gently at 50°C for 200 min. And then the extracted filtrates were collected by centrifuging at 10,000 rpm for 10 min and filtering with filter paper. The resulting extracted filtrate was vacuum-concentrated (Vacufuge concentrator, RE-2000, Shanghai Yarong Biochemical Instrument factory, China) and dialyzed using MWCO 100 dialysis bags (Nanjing Dulai biotechnology Co., Ltd., China) at 4°C for 12 h. Finally, the desalted filtrate was lyophilized to constant weight and weighed. The broth of bio-converted product and the filtrates of the control SPW medium were concentrated, dialyzed, lyophilized and weighed, too. Both the dried powder of the mycelia extracts and fermented broth were dissolved in distilled water to obtain water extracts for determination of their ACE inhibitory activity.

ACE inhibitory assay of the water extracts: The ACE-inhibitory activity of water extracts from products of *X. badius* bio-converted SPW and fermented product in PDA were determined with the RP-HPLC method mentioned by Hyun and Shin (2000) with minor changes. Chemicals used in the assay included ACE from rabbit lung (Sigma, St. Louise MO, USA, dissolved in DDW at the concentration of 250 mU mL^{-1}), Hippuryl-L-histidyl-L-

leucine (HHL, Sigma, St. Louise MO, USA, dissolved in HEPES buffer at 6.5 mM) and HEPES buffer (50 mM, pH 8.3, containing 360 mM NaCl). Sample solutions of water extracts from primary bioconversion were prepared in various concentrations by dissolving in 50 mM HEPES buffer. Sample solutions of water extracts from optimization experiments were all prepared the concentration of 0.400 mg mL^{-1}. The steps of the assay were show in Table 1.

The enzyme activity of ACE was evaluated by determining the amount of hippuric acid (Hip) liberated in the assay by RP-HPLC on a Varian C18 column (4.6×150 mm, 5u, Eka Nobel, Sweden). After 20 μL of reaction product being injected, the column was eluted with the mobile phase (contained percentage acetonitrile: water = 30:70, v/v, containing 0.2% acetic acid) at the 1.0 mL min^{-1} of flow rate. The detection wavelength was 228 nm. ACE inhibitory rate was formulated by the Eq. 1:

$$\text{Inhibitory rate (\%)} = \frac{HA_C - HA_S}{HA_C - HA_B} \times 100\% \quad (1)$$

where, HA_C refers to the area of hippuric acid peak of control, HA_S to sample and HA_B to blank group, respectively. The IC_{50} value was calculated as the concentration of a certain sample required to reduce the enzyme activity of ACE (area of the hippuric acid peak) by 50%.

Table 1: Steps of the ACE inhibitory assay

Reagents (μL)	Control	Blank	Samples
ACE	30	30	30
HEPES buffer	30	30	
Samples			30
HCl (1 M)		100	
Incubated at 37°C for 5 min			
HHL	90	90	90
Incubated at 37°C for 30 min			
HCl (1 M)	100		100

Bioconversion conditions optimization for mycelium biomass and ACE inhibitory activity: Bioconversion conditions were optimized with Plackett-Burman design, path of steepest ascent and response surface methodology sequencely.

Plackett-Burman (PB) design: A total of 8 independent variables which were named as X1-8, were screened in PB design for finding out the significant variables which influence the mycelium biomass and the ACE inhibitory rate of water extract from the mycelium. Each independent variable was set a high (+1) and low (-1) level. In the present study, the low (-1) levels were same as those in initial bioconversion, the high (+1) levels were 1.25 times of the low ones. Variable A, B and C served as dummy columns to calculate the standard error. All experimental trials, factors and their levels were shown in Table 2.

The biomass and the ACE inhibitory rate were their response values. Based on the results of the PB design, the first-order model was:

$$Y_{(1,2)} = \beta_0 + \sum \beta_i X_i \ (i = 1....k) \quad (2)$$

where, Y_1 was the mycelium biomass and Y_2 the ACE inhibitory activity of water extracts from the mycelium; β_0 was the constant; β_i were the regression coefficients; X_i were the actural independent factors shown in Table 2.

Path of steepest ascent: After the key variables being chosen in terms of the results of the PB design, the steepest ascent experiment was preceded by the key factors moving sequentially along the path of steepest ascent or descent for moving rapidly to the optimum region. The key factors and their levels of experimental trials (8 in all) in the path of speed ascent were shown in Table 3.

Table 2: Experimental trails and their responses in Plackett-Burman experimental design

	Experimental factors												
Runs	X_1	X_2	A	X_3	X_4	B	X_5	X_6	C	X_7	X_8	Mycelium biomass (g L^{-1})	ACE inhibitory rate (%)
1	-1(6.5)	-1(5.0)	-1	-1(100)	-1(5)	-1	-1(120)	-1(1.30)	-1	-1(3)	-1(25)	7.995±0.605	50.495±0.115
2	1(8.5)	1(6.5)	-1	-1(100)	-1(5)	1	-1(120)	1(1.65)	1	-1(3)	1(30)	15.343±0.778	67.779±0.156
3	-1(6.5)	-1(5.0)	-1	1(125)	-1(5)	1	1(150)	-1(1.30)	1	1(5)	1(30)	2.987±0.350	25.003±0.169
4	-1(6.5)	1(6.5)	-1	1(125)	1(10)	-1	1(150)	1(1.65)	1	-1(3)	-1(25)	1.176±0.305	20.495±0.148
5	-1(6.5)	-1(5.0)	1	-1(100)	1(10)	1	-1(120)	1(1.65)	1	1(5)	-1(25)	1.389±0.372	25.003±0.128
6	-1(6.5)	1(6.5)	1	1(125)	-1(5)	-1	-1(120)	1(1.65)	-1	1(5)	1(30)	2.045±0.378	27.779±0.400
7	-1(6.5)	1(6.5)	1	-1(100)	1(10)	1	1(150)	-1(1.30)	-1	-1(3)	1(30)	10.931±0.556	45.596±0.281
8	1(8.5)	1(6.5)	-1	1(125)	1(10)	1	-1(120)	-1(1.30)	-1	1(5)	-1(25)	19.509±0.824	72.360±0.192
9	1(8.5)	-1(5.0)	1	1(125)	-1(5)	1	1(150)	1(1.65)	-1	-1(3)	-1(25)	12.192±0.670	57.032±0.092
10	1(8.5)	1(6.5)	1	-1(100)	-1(5)	-1	1(150)	-1(1.30)	1	1(5)	-1(25)	20.804±0.877	77.718±0.242
11	1(8.5)	-1(5.0)	-1	-1(100)	1(10)	-1	1(150)	1(1.65)	-1	1(5)	1(30)	11.190±0.760	65.968±0.142
12	1(8.5)	-1(5.0)	1	1(125)	1(10)	-1	-1(120)	-1(1.30)	1	-1(3)	1(30)	14.699±0.802	70.192±0.389

X_1: Solid to liquid ratio (%, m/v), X_2: Bran powder content (%), X_3: Volume of liquid medium (mL), X_4: Inoculation amount of mycelium (%), X_5: Rotation rate (rpm), X_6: Concentration of glacial acetic acid (%), X_7: Cultural temperature (°C), X_8: Cultivation time (h), A, B and C refer to dummy columns

Table 3: Experimental trails and their responses in the path of steepest ascent (descent)

	Experimental factors				
Trials	X_1	X_2	X_6	Mycelium biomass (g L^{-1})	ACE inhibitory rate (%)
1	7.50	5.50	1.50	13.485±0.167	27.951±0.303
2	8.50	6.00	1.40	17.684±0.717	31.900±2.021
3	9.50	6.50	1.30	21.603±0.311	43.476±0.229
4	10.50	7.00	1.20	26.160±0.989	65.670±0.465
5	11.50	7.50	1.10	27.376±0.814	58.060±0.095
6	12.50	8.00	1.00	28.554±0.673	52.063±0.343
7	13.50	8.50	0.90	25.677±0.584	45.154±0.315
8	14.50	9.00	0.80	22.165±0.271	33.725±0.452

Table 4: Experimental trails and their responses in CCD

	Experimental factors				
Trails	X_1	X_2	X_6	Mycelium biomass (g L^{-1})	ACE inhibitory rate (%)
1	0(11.50)	0(7.50)	0(1.10)	28.998±0.177	67.233±0.344
2	1(13.50)	-1(6.50)	-1(0.90)	20.529±0.603	36.429±0.490
3	-1(9.50)	-1(6.50)	1(1.30)	15.116±0.064	38.779±0.524
4	0(11.50)	0(7.50)	0(1.10)	27.115±0.483	67.879±0.448
5	1.68(14.86)	0(7.50)	0(1.10)	25.652±0.200	52.189±0.699
6	1(13.50)	1(8.50)	1(1.30)	23.814±0.499	62.861±0.570
7	-1(9.50)	1(8.50)	-1(0.90)	17.505±0.359	42.654±0.145
8	1(13.50)	-1(6.50)	1(1.30)	14.878±0.442	57.582±0.549
9	0(11.50)	-1.68(5.82)	0(1.10)	23.730±0.219	44.002±0.308
10	-1(9.50)	1(8.50)	1(1.30)	16.257±0.767	37.663±0.721
11	0(11.50)	0(7.50)	0(1.10)	25.648±0.316	68.768±0.393
12	-1(9.50)	-1(6.50)	-1(0.90)	15.538±0.608	34.662±0.287
13	0(11.50)	0(7.50)	0(1.10)	26.017±0.425	66.336±0.558
14	0(11.50)	0(7.50)	0(1.10)	26.593±0.310	69.922±0.447
15	0(11.50)	0(7.50)	0(1.10)	29.514±0.544	65.927±0.605
16	0(11.50)	0(7.50)	-1.68(0.76)	18.951±0.240	49.100±0.464
17	-1.68(8.14)	0(7.50)	0(1.10)	10.031±0.192	33.603±0.319
18	1(13.50)	1(8.50)	-1(0.90)	27.098±0.845	46.828±0.433
19	0(11.50)	1.68(9.18)	0(1.10)	29.345±0.393	50.712±0.612
20	0(11.50)	0(7.50)	1.68(1.44)	13.912±0.176	59.185±0.122

The base point of the path of steepest ascent was the centre of the PB design. The step-size of it was decided by experimenter based on process knowledge or other practical consideration. The direction of steepest ascent (descent) was the direction in which the response increased most rapidly. The levels of factors except the key ones were fixed according to the change trend in PB design.

Response Surface Methodology (RSM): A Central Composite Design (CCD) experimental plan with the key factors at 5 levels, 20 experimental trials in all (Table 4), was used in RSM. It was conducted in the optimum vicinity (fixed through the path of steepest ascent) to locate the true optimum point of the the key factors selected by PB design.

Results of the CCD were fit with a second-order polynomial equation:

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

where, Y_1 and Y_2 refer to the myecelium biomass and ACE inhibitory rate of the water extract, β_0 to the offset term, β_i to the linear effects, β_{ii} to the squared terms, β_{ij} to the interaction effects, X_i and X_j to the independent factors showed in Table 4.

Verification of the model: The model adequacy was tested by experiments at optimum condition in order to experimentally obtain a maximum mycelium biomass and ACE inhibitory activity suggested in RSM. Then validity of the models were evaluated by comparing results with values obtained in initial bioconversion stage and the predicted values.

Statistical analysis: All experiments in the present study were carried out in triplicate. Results of initial bioconversion stage, the steepest ascent and verification experiments were statistically analyzed by analysis of variance (ANOVA) and t-test with SPSS 16.0 software. The PB and RSM experimental design and statistical analysis were done with the design expert 7.1.6 software.

RESULTS AND DISCUSSION

Results of the bioconversion before optimization: Results of primary liquid bioconversion showed that mycelium of *X. badius* could grow well in the medium made up of SPW (Fig. 1). The mycelium biomass (dried weight) reached to 20.903±0.062 g L^{-1} after 3 days bioconversion, were significantly higher than that from PDA (3.697±0.274 g L^{-1}, $p<0.001$). Yields of water extracts from the result mycelium and broth of bioconversion was measured as 8.631±0.194 and 15.528±0.818 g L^{-1}, respectively which were significantly higher than that from PDA (1.371±0.082 and 10.893 g L^{-1}±1.026, $p<0.001$). The ACE inhibitory IC_{50} of water extracts from the result mycelium of bioconversion was 0.400±0.000 mg mL^{-1} which was significantly lower than that from the fermented broth of bioconversion and mycelium culured in PDA (1.014±0.009 and 3.654±0.034 mg mL^{-1}, respectively $p<0.01$ and $p<0.001$). However, the ACE inhibitory IC_{50} of water extracts from the fermented broth of PDA (0.179±0.003) was lower significantly than that of above all ($p<0.001$, $p<0.01$ or $p<0.05$). The water extracts from the result mycelium of bioconversion relatively higher ACE inhibitory activity and was considered as the desired product in futher study for optimization.

PB design for screening of bioconversion conditions: The total 8 factors were screened using PB statistical experimental design, the mycelium biomass in bioconversion and ACE inhibitory rate of water extracts from the mycelium were chosen as their response variables. The mycelium biomass of *X. badius* ranged from 1.176±0.305 to 20.804±0.877 g L^{-1} and the ACE inhibitory rate of water extract from the mycelium was from 20.495±0.148 to 77.718±0.242% (Table 2). The first-order models of the biomass and ACE inhibitory activity of water extracts of actual factors were established based on the results which are as follow:

$$Y_1 = -12.018+5.601X_1+2.151X_2-1.254X_3 -0.082X_4-0.009X_5-15.995X_6-0.368X_7-0.195X_8 \quad (4)$$

$$Y_2 = -7.657+18.056X_1+2.004X_2-4.975X_3 -0.206X_4-0.121X_5-36.814X_6-1.480X_7-0.026X_8 \quad (5)$$

where, Y_1 refers to the mycelium biomass, Y_2 the ACE inhibitory activity, X_{1-8} were the actural factors shown in Table 2.

The results of regression and ANOVA analysis of PB design for the mycelium biomass (Table 5) showed that there were 3 of the 8 factors had a significant effect on the biomass with $p<0.05$. Furthermore, the pareto plot (Fig. 2)

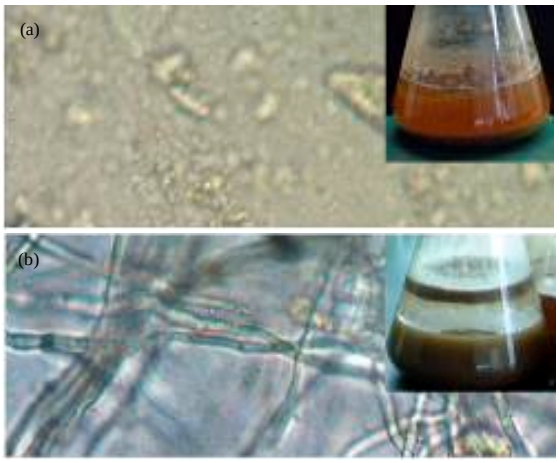

Fig. 1(a-b): Mycelia of *Xerocomus badius* growing in liquid SPW medium, (a) Control medium and (b) Mycelia of *Xerocomus badius* in shaking flasks and magnified to 400x

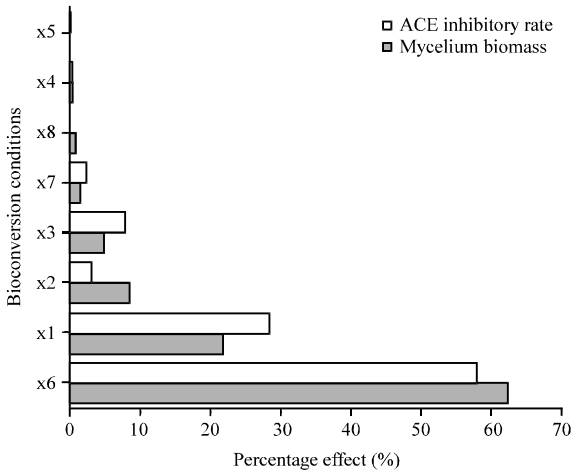

Fig. 2: Pareto plot for Plackett-Burman parameter estimates for independent factors

illustrated the order of significance of the factors affecting the biomass which was $X_6>X_1>X_2>X_3>X_7> X_8>X_4>X_5$. In ANOVA analysis of PB design, the Model F-value of 26.192 implied that the model was significant ($p = 0.0107$). The determination coefficient (R^2) of the regression model for the mycelium biomass (0.9859) meant that 98.59% of the variability of the response was captured by the model. The adjusted determination coefficient (R^2_{Adj}), was calculated as 0.9482 which indicated a high degree of goodness-of-fit of regression equations. These results implied that the model could be used to navigate the design space.

Table 5: ANOVA analysis for the Plackett-Burman Factorial Model of mycelium biomass and ACE inhibitory rate

	Mycelium biomass (g L^{-1})					ACE inhibitory rate (%)				
Sources	Sum of squares	df	Mean square	F-value	p-value	Sum of squares	df	Mean square	F -value	p-value
Model	525.827	8	65.728	26.192	0.0107	4803.670	8	600.459	14.527	0.0250
X1	376.474	1	376.474	150.021	0.0012	3912.398	1	3912.398	94.654	0.0023
X2	31.227	1	31.227	12.443	0.0387	27.104	1	27.104	0.656	0.4773
X3	18.864	1	18.864	7.517	0.0712	296.989	1	296.989	7.185	0.0750
X4	0.510	1	0.510	0.203	0.6828	3.195	1	3.195	0.077	0.7990
X5	0.241	1	0.241	0.096	0.7771	39.593	1	39.593	0.958	0.3999
X6	94.019	1	94.019	37.466	0.0088	498.059	1	498.059	12.050	0.0403
X7	1.623	1	1.623	0.647	0.4801	26.280	1	26.280	0.636	0.4835
X8	2.871	1	2.871	1.144	0.3632	0.052	1	0.052	0.001	0.9741
Residual	7.528	3	2.509			124.001	3	41.334		
Cor total	533.356	11				4927.671	11			
		$R^2 = 0.9859$, $R^2_{Adj} = 0.9482$					$R^2 = 0.9748$, $R^2_{Adj} = 0.9077$			

The results of regression and ANOVA analysis of PB design for the ACE inhibitory rate of the water extract (Table 5) implied that there were 2 of the 8 factors had a significant effect on the ACE inhibitory rate with $p<0.05$. Furthermore, the pareto plot (Fig. 2) illustrated the order of significance of the factors affecting the ACE inhibitory activity which was $X_1>X_6>X_3>X_5>X_2>X_7>X_4>X_8$. The Model F-value of 14.527 implied the model was significant ($p = 0.0250$). The R^2 value of the regression model of the ACE inhibitory activity (0.9748) meant that 97.48% of the variability of the response was captured by the model. The R^2_{Adj} of 0.9077 indicated a high degree of goodness-of-fit of regression equations. These results implied that the model could be used to navigate the design space.

In some reports, factors with confidence level above 80% (Pujari and Chandra, 2000) or 85% (Xiong *et al*., 2004) were chosen as key factors for further optimization. There were 3-5 significant factors chosen as major factors in others (Abdel-Fattah *et al*., 2005; Chen *et al*., 2005). In this study, variables of X_6, X_1, X_2 in the model for mycelium biomass and X_1 and X_6 in the model for ACE inhibitory rate were significant. So, X_1, X_2 and X_6 (with confidence level of 92.559 and 89.309% in model for biomass and ACE inhibitory rate, respectively) were considered as major factors in further optimization when both of mycelium biomass and ACE inhibitory rate were kept in mind. Other variables were not selected for the following optimization because of their less significant effect used in all trials at their high (-1) level because their regression coefficients were negative in the two models.

Path of steepest ascent for locating the region of optimum response: Based on first-order models, the path of steepest ascent was done to find the proper region of maximum mycelium biomass and ACE inhibitory rate. The path of steepest ascent started from the centre of the factorial design and moved along the path in which the solid to liquid radio and content of bran powder were increasing, whereas the concentration of glacial acetic acid was decreasing.

The results of the path of steepest ascent experiments (Table 3) indicated that the optimum neighborhood of mycelium biomass near to 28.55367 g L^{-1} when the solid to liquid ratio was of 12.50%, the content of bran powder of 8.00% and concentration of glacial acetic acid was 1.00%. The optimum neighborhood of the ACE inhibitory rate near to 65.67039% when the solid to liquid radio was 10.50%, the content of bran powder was 7.00% and concentration of glacial acetic acid was 1.20%. According to these results, the levels of three key factors in CCD were defined as 9.50% (-1 level), 11.50% (0 level), 13.50% (1 level) of the solid to liquid radio, 6.50% (-1 level). About 7.50% (0 level), 8.50% (1 level) of the content of bran powder and 0.90% (-1 level), 1.10% (0 level), 1.30% (1 level) of concentration of glacial acetic acid (Table 4) which included the optimum neighborhood of mycelium biomass and ACE inhibitory rate.

Central Composite Design (CCD) for further optimization: Three variables (X_1, X_2 and X_6) were used to determine the optimum values by response surface methodology via CCD. It was indicated (Table 4) that variation in the mycelium biomass and ACE inhibitory rate depending upon the cultural conditions was considerable. The results of the CCD were fit with the following second-order models in terms of actual factors:

$$Y_1 = -222.430+18.922X_1-0.629X_2+235.145X_6+0.775\ X_1X_2 -2.270X_1\ X_6+0.963X_2X_6-0.890X_1^2-0.486\ X_2^2-101.747X_6^2 \quad (6)$$

$$Y_2 = -777.618+38.889X_1+120.867X_2+238.893X_6+ 0.550X_1X_2+11.894X_1X_6-8.893X_2X_6-2.310X_1^2-7.662\ X_2^2-131.579X_6^2 \quad (7)$$

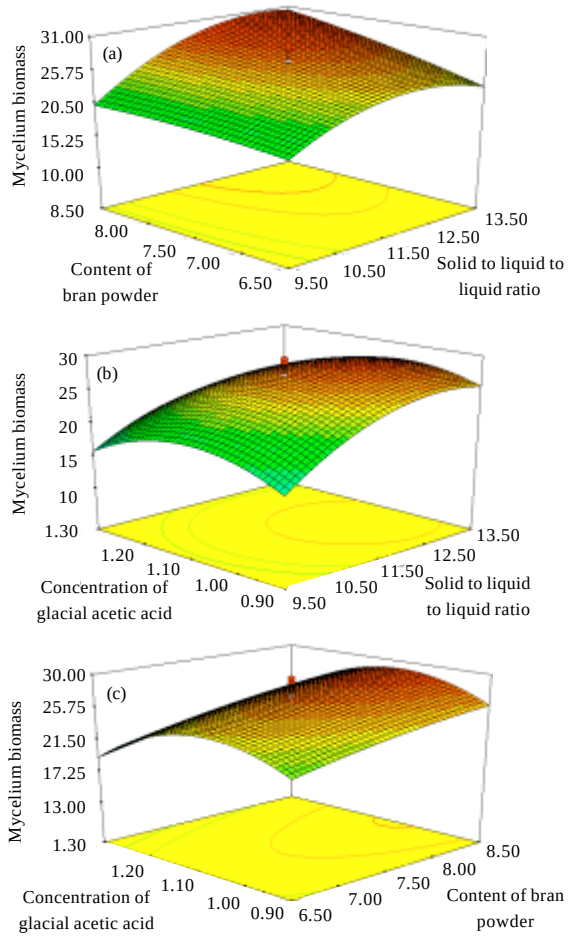

Fig. 3(a-c): Response surface contour of the model for the mycelium biomass of *X. badius* grown in liquid SPW medium, Interaction of the solid to liquid ratio and (a) Bran powder content, (b) Concentration of glacial acetic acid and (c) Interaction of the bran powder content and the concentration of glacial acetic acid

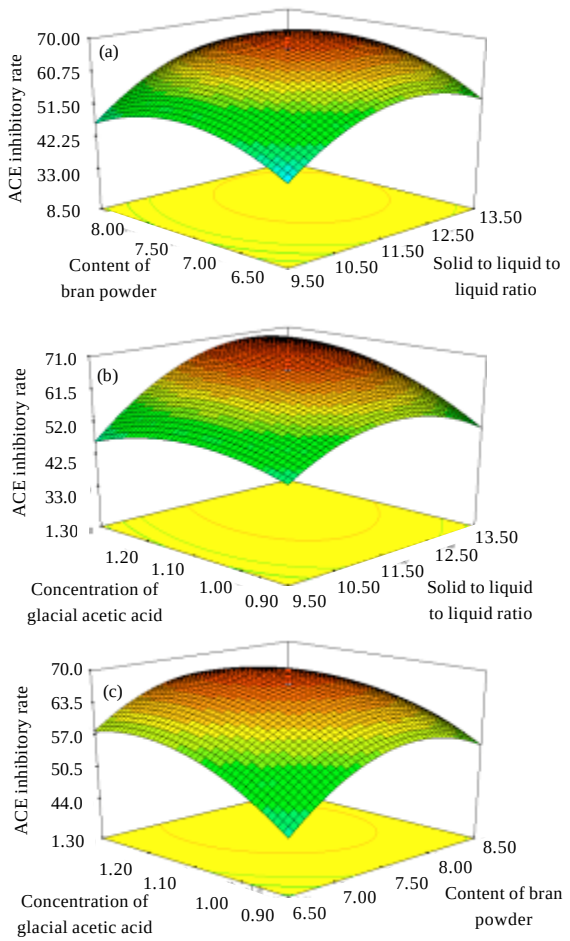

Fig. 4(a-c): Response surface contour of the model for ACE inhibitory rate of water extract from the mycelium of *X. badius* grown in liquid SPW medium, (a) Interaction of the solid to liquid ratio and the bran powder content, (b) Interaction of the solid to liquid ratio and the concentration of glacial acetic acid and (c) Interaction of the bran powder content and the concentration of glacial acetic acid

The relationships between the dependent and independent variables were shown by Eq. 6 and 7. According to the results of regression and ANOVA analysis of the models of CCD (Table 6), the Models F-value of 23.980 and 82.662 implied that the models were significant ($p<0.0001$). The values of adjusted determination coefficients (R^2_{Adj}) for the two regression models were 0.9159 and 0.9748 which meant a high degree of correlation between the experimental and predicted values. The p-values of the lack of fit for the two regression models (0.3544 and 0.1530) were higher than 0.05 which implied that the lack of fit were not significant and the models could describe variability of the mycelium biomass and ACE inhibitory rate effected by the factors successfully.

The three-dimensional response surface plots were constructed with Design-Expert 7.1.6 software to estimate the effects of the factors and their interactions on the mycelium biomass (Fig. 3a-c) and ACE inhibitory rate (Fig. 4a-c). As shown in Fig. 3a-c, the mycelium biomass rose up with the solid to liquid ratio ranged from 9.50% to around 13.00% and declined with the solid to liquid ratio

Table 6: ANOVA analysis for quadratic regression models for mycelium biomass and ACE inhibitory rate in CCD

	Mycelium biomass (g L^{-1})					ACE inhibitory rate (%)				
Sources	Sum of squares	df	Mean square	F-value	p-value	Sum of squares	df	Mean square	F-value	p-value
Model	663.46100	9	73.718000	23.980	<0.0001	3087.763	9	343.0850	82.662000	<0.0001
X1	169.94300	1	169.943000	55.282	<0.0001	482.800	1	482.8000	116.324000	<0.0001
X2	57.64100	1	57.641000	18.751	0.0015	83.848	1	83.8480	20.202000	0.0012
X6	26.65300	1	26.653000	8.670	0.0147	207.817	1	207.8170	50.071000	<0.0001
X1X2	19.21200	1	19.212000	6.250	0.0315	9.685	1	9.6850	2.333000	0.1576
X1X6	6.59800	1	6.598000	2.146	0.1736	181.080	1	181.0800	43.629000	<0.0001
X2X6	0.29700	1	0.297000	0.097	0.7624	25.307	1	25.3070	6.097000	0.0331
X12	182.67600	1	182.676000	59.424	<0.0001	1230.214	1	1230.2140	296.404000	<0.0001
X22	3.40100	1	3.401000	1.106	0.3177	846.080	1	846.0800	203.852000	<0.0001
X62	237.42600	1	237.426000	77.234	<0.0001	399.205	1	399.2050	96.183000	<0.0001
Residual	30.74100	10	3.074000			41.505	10	4.1505		
Lack of fit	18.04800	5	3.610000	1.422	0.3544	30.177	5	6.0350	2.664143	0.1530
Pure error	12.69281	5	2.538562			11.327	5	2.2650		
Cor Total	694.20170	19				3129.267	19			
		$R^2 = 0.9557$, $R^2_{Adj} = 0.9159$					$R^2 = 0.9867$, $R^2_{Adj} = 0.9748$			

ranged from about 13.00-13.50%. The effect of content of bran powder and concentration of glacial acetic acid on the mycelium biomass also exhibited sensitivity within the tested range. The optimal conditions predicted for the highest mycelium biomass of *X. badius* grown in liquid SPW medium were final the solid to liquid radio was of 12.985%, content of bran powder of 8.50% and the concentration of glacial acetic acid of 1.054%. As a result, the highest mycelium biomass was predicted as 30.940 g L^{-1}.

Figure 4a-c illustrated the interaction between the three factors corresponding to the ACE inhibitory rate of water extract from the mycelium. The elliptical contours indicated that the ACE inhibitory rate increased with increasing of solid to liquid ratio up to 9.50-12.50% but decreased rapidly beyond this. The effect of content of bran powder and concentration of glacial acetic acid on the ACE inhibitory rate also exhibited sensitivity within the tested range. The optimum cultural condition for the highest ACE inhibitory rate was predicted as the solid to liquid ratio of 12.449%, content of bran powder of 7.631% and the concentration of glacial acetic acid of 1.213%. As a result, ACE inhibitory rate was predicted as 70.421%.

Finally, the model predicted that the optimal cultural conditions were the solid to liquid radio was of 12.421%, content of bran powder of 7.964% and the concentration of glacial acetic acid of 1.117%, as far as both of mycelium biomass and ACE inhibitory rate were concerned. Under the optimal conditions, the mycelium biomass and ACE inhibitory rate were predicted as 29.222 g L^{-1} and 68.669%.

Validation experiments: Experiments were carried out in triplicate using the optimum cultural conditions predicted, respectively for the highest mycelium biomass, the highest ACE inhibitory rate and both for the highest mycelium biomass and ACE inhibitory rate obtained from CCD in order to validate the suitability of the regression models for predicting optimum response values. Under the optimal cultural conditions for the highest mycelium biomass, the measured mycelium biomass was determined as 30.244±0.213 g L^{-1} which was near to the predicted value and enhanced by 44.687% compared with the mycelium biomass gained before optimization. Under the optimal cultural conditions for the highest ACE inhibitory rate, the measured value was determined as 71.057±0.534% which was very near to the predicted one and improved by 42.144% compared with the ACE inhibitory rate gained before optimization. Under the optimal cultural conditions both for the highest mycelium biomass and ACE inhibitory rate, the measured values were determined as 28.890±1.011 g L^{-1} and 69.747±0.843% which were also very near to the predicted values and improved by 38.210 and 38.948%, respectively compared with the values gained before optimization.

Furthermore, under the optimal cultural conditions both for the highest mycelium biomass and ACE inhibitory rate, the yield of water extract from the mycelium was culculated as 13.926 g L^{-1} and was improved by 44.596% compared with the yield gained before optimization. The ACE inhibitor IC_{50} value was determined as 0.212 mg mL^{-1} and lower than the value gained befoe optimization significantly. Besides, the score of deodorization of the bioconversion under the optimal cultural conditions both for the highest mycelium biomass and ACE inhibitory rate were determined as 3.556±0.063 which was a little bit improved but not diffirent siginificantly from the score gained before optimization.

The combination of Plackett-Burman, path of steepest ascent and CCD was effective and reliable in selecting the statistically significant factors and locating the optimal levels of those factors for the highest mycelium biomass and ACE inhibitory rate of water extract from mycelium of *X. badius* grown in liuquid SPW medium

in this study. The SPW contains large amounts of nutritive components, mineral calcium and phosphorus (Heu *et al*., 2003). It had been fermented by lactic acid bacteria for recovering chitosan and chitin (Rao and Stevens, 2005; Phuvasate and Su, 2010; Cira *et al*., 2002). But the SPW is subalkline (Rao and Stevens, 2005; Phuvasate and Su, 2010), so that glacial acetic acid (about 1%) has been added into when it being fermented. The acidic mediums (pH 5.50-6.50) are more suitable for mycelia of many kinds of edible/medicinal fungi growth in liquid fermentation (Park *et al*., 2001; Shih *et al*., 2007; Kim *et al*., 2005). In the present study, 1.117% of glacial acetic acid was added to the medium for the highest mycelium biomass and ACE inhibitory rate when the solid to liquid radio was 12.421%, the pH value of which was determined as 5.831±0.113 which was in the range of optimal pH value for most of fungi. Besides, there has not been any report about the optimal pH value for ACE inhibitory rate of water extract from mycelium which was determined as 5.671±0.226 in the present study when the concentration of glacial acetic acid and the solid to liquid radio were 1.117 and 12.449%.

SPW and its extracts have an unpleasant fishy odor which limits their reutilization. Thus, treatment for deodorization is a extremely important point in its recycling. Biological deodorization has increasingly become a considered method for odor control which can eliminating the fishy odor permanently without the secondary pollution at a low cost. Currently, yeasts are always the stains used for deodorization (Yan *et al*., 2013). A edible/medicinal fungal stain, *X. badius*, was proved to be effective in deodorization of SPW in the process liquid fermentation for mycelium biomass and ACE inhibitor, for the first time, in this study. This result has vast importance to recycle the wastes with unpleasant odors. It is worth to explore the mechanism for deodorization effect of bioconversion with *X. badius* and conditions for the best effect of bioconversion in further study.

CONCLUSION

The main factors affect on the mycelium biomass and ACE inhibitory rate of water extract from the mycelium of *X. badius* cultured in SPW medium and their optimum level were screened for the first time. The solid to liquid ratio, content of bran powder and concentration of glacial acetic acid were chosen as factors had significant effect on mycelium biomass and ACE inhibitory rate and the optimum level of them were located as 12.421, 7.964 and 1.117%, respectively. Under the optimum conditions, mycelium biomass was measured as 28.890 g L^{-1} and improved by 38.210% compared to that before optimization. The ACE inhibitory rate of the water extract from the mycelium was culculated as 69.746% and improved by 38.948% compared to that before optimization. SPW can be deodorized effectively after being converted by *X. badius*. Further purification and characterization of the ACE inhibitory substance are on the march to investigate the hypertensive activity, character and structure of the ACE inhibitor from the mycelium of *X. badius* grown in the SPW medium. This study affords a experimental and theoretical foundation for low-cost reusing SPW and producing ACE inhibitor in industrial scale. in industrial scale.

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