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Pineapple cannery waste as a potential substrate for microbial biotranformation to produce vanillic acid and vanillin

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Abstract

In this study, pineapple cannery waste materials were used as substrate for the microbial production of vanillic acid and vanillin by *Aspergillus niger* I-1472 and *Pycnoporus cinnabarinus* MUCL 39533. Biotransformation of ferulic acid from pineapple waste by *A. niger* I-1472 to vanillic acid was optimized using Response Surface Methodology (RSM). A central composite rotatable design was used to allocate treatment combinations and factors tested for their influence on vanillic acid production were inoculum size, yeast extract concentration, diammonium tartrate concentration and initial medium pH. The amount of vanillic acid produced was used as the response for the fermentation study and was assumed to be under the influence of the four factors tested. The estimated conditions for optimal vanillic acid production were inoculum size, 3.08 $\times 10^5$ CFU mL^{-1}; yeast extract, 0.37 gL^{-1}; diammonium tartrate, 3.88 gL^{-1} and initial pH, 4.3. Subsequent biotransformation of vanillic acid by *P. cinnabarinus* MUCL 39533 to vanillin was enhanced with the addition of resin. Under these optimal conditions, 141.00 mgL^{-1} of vanillin was produced from 5 g of pineapple cannery waste.

Introduction

The pineapple is an important member of the international fruit sector, with production growing from 4 million tons in 1960 to 16 million tons in 2005. Pineapples can be exported in its fresh or processed forms, namely, canned pineapple, pineapple pulp and juice (FruiTrop, 2008). Leading producer countries are located in Asia (China, India, Indonesia, the Philippines, Thailand and Vietnam), Latin America (Brazil, Colombia, Costa Rica and Mexico) and Africa (Kenya and Nigeria). The fresh pineapple trade accounts for about 11% of the total production while the juice and canned fruit trade makes up 32% (FruiTrop, 2007).

In Malaysia, the pineapple industry is the oldest agro-based export-oriented industry, dating back to the 19th century. Although it can be considered as relatively small compared to palm oil and rubber, the pineapple industry plays an important role in the country's socio-economic development. In 2011, 17,165 metric tons of canned pineapple was produced in Malaysia. Currently, 95% of the canned pineapple produced in Malaysia is for export and the remaining 5% is for domestic consumption (MPIB, 2011). During pineapple processing, the stem, crown, core and peel are removed. As much as 30 - 50% of the total fruit weight is discarded as waste during canning. Therefore, the pineapple canning industry produces a substantial amount of waste comprising solid and liquid waste. At the cannery, the peel, core and unwanted fruits are crushed, after which this solid waste is used as low-cost animal feed and the liquid waste is sent for alcohol fermentation. Waste disposal can be problematic because the waste material, being high in moisture and sugar content, is prone to microbial spoilage.

As pineapple waste contains high levels of carbohydrates which can be used as a source of carbon for microbial fermentation, it is a promising substrate for bioconversion into useful value-added products. According to Tilay *et al.* (2008), pineapple peels contain ferulic acid, which can act as a precursor for vanillic acid and vanillin production. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the main component in vanilla flavor extracted from vanilla beans. It is a highly sought-after flavor compound and is widely used in the food, confectionery, perfumery, cosmetics and pharmaceutical industries. High demand for natural flavors from consumers has triggered research in natural vanillin production from natural resources through microbial biotransformation (Priefert *et al*., 2001). Lesage-Meessen *et al*. (1996) reported the biotransformation of ferulic acid to vanillin in a two-step process involving two filamentous fungi. In the first step, ferulic acid was metabolized to vanillic acid by the ascomycete, *Aspergillus niger* and subsequently, the vanillic acid was reduced to vanillin by the basidiomycete, *Pycnoporus cinnabarinus*.

In this study, pineapple cannery waste, namely pineapple peel and core, were used as ferulic acid

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source to produce vanillic acid and vanillin by fermentation. The two-step approach as described by Lesage-Meessen *et al.* (1996) was used in this study. The aims of this research were to optimize the biotransformation of ferulic acid from pineapple wastes by *A. niger* I-1472 to vanillic acid and subsequently to vanillin by *P. cinnabarinus* MUCL 39533.

Materials and Methods

Raw material

Mature pineapple (*Ananas comosus*) of Gandul cultivar was obtained from Malaysian Pineapple Industry Board. The Maturity Index established by the Malaysian Pineapple Industry Board for Gandul cultivar was used to evaluate the maturity of the pineapple samples.

Microbial strains

Aspergillus niger I-1472 and *P. cinnabarinus* MUCL 39533 were obtained from Institut Pasteur, France and Mycotheque de l'Universite catholique de Louvain, Belgium, respectively. The strains were stored at -20°C in 10% (v/v) glycerol (BDH Laboratory Supplies, UK).

Aspergillus niger I-1472

For inoculum preparation, the strain was cultured on Potato Dextrose Agar (PDA) (Merck, Germany) at 26°C. Spores obtained from 4-day-old culture were dislodged with an L-shaped glass rod by adding 10 mL sterile distilled water to the culture in order to create a spore suspension. This spore suspension was used as the master suspension, which was appropriately diluted with sterile distilled water to the required density of spores.

Pycnoporus cinnabarinus MUCL 39533

The strain was cultured on Malt Extract Agar (MEA) (Merck, Germany) for 4 days at 30°C. Ten mL sterile distilled water was added to the 4-day old culture and the spores were gently dislodged with the use of a L-shaped glass rod to form a spore suspension. One mL of this spore suspension was then inoculated into 100-mL basal medium proposed described by Stentelaire *et al*. (2000) containing (g L^{-1}) maltose, 20; diammonium tartrate, 1.82; KH_2PO_4, 0.2; $CaCl_2.2H_2O$, 0.00132; $MgSO_4.7H_2O$, 0.5; yeast extract, 0.5. Thiamine hydrochloride (0.0025 gL^{-1}) was added to this medium as described by Gross *et al.* (1990). This culture was incubated at 30°C for 10 days during which sporulation occurred. At day-10, the cultivation medium was poured into a sterile Waring blender and blended to obtain mycelium fragments. Five mL of this mycelium fragments was used as inoculum for fermentation.

Table 1. Treatment combinations and responses

Inoculum size *A.niger* I-1472 log(CFU mL^{-1}), X_1	Yeast extract (g L^{-1}) , X_2	Diammonium tartrate (g L^{-1}), X_3	Initial pH, X_4	Response[a] (mg L^{-1}), Y
5.041[b] (-1)[c]	0.5 (-1)	2.13 (-1)	5.5 (-1)	46.88
5.041 (-1)	0.5 (-1)	2.13 (-1)	8.5 (1)	65.06
5.041 (-1)	0.5 (-1)	3.38 (1)	5.5 (-1)	100.87
5.041 (-1)	0.5 (-1)	3.38 (1)	8.5 (1)	76.41
5.041 (-1)	1.5 (1)	2.13 (-1)	5.5 (-1)	84.08
5.041 (-1)	1.5 (1)	2.13 (-1)	8.5 (1)	96.54
5.041 (-1)	1.5 (1)	3.38 (1)	5.5 (-1)	72.07
5.041 (-1)	1.5 (1)	3.38 (1)	8.5 (1)	47.65
6.041 (1)	0.5 (-1)	2.13 (-1)	5.5 (-1)	57.31
6.041 (1)	0.5 (-1)	2.13 (-1)	8.5 (1)	56.62
6.041 (1)	0.5 (-1)	3.38 (1)	5.5 (-1)	96.55
6.041 (1)	0.5 (-1)	3.38 (1)	8.5 (1)	43.11
6.041 (1)	1.5 (1)	2.13 (-1)	5.5 (-1)	119.76
6.041 (1)	1.5 (1)	2.13 (-1)	8.5 (1)	116.44
6.041 (1)	1.5 (1)	3.38 (1)	5.5 (-1)	88.14
6.041 (1)	1.5 (1)	3.38 (1)	5.5 (1)	42.85
6.301 (2)	1.0 (0)	2.75 (0)	7.0 (0)	83.22
4.301 (-2)	1.0 (0)	2.75 (0)	7.0 (0)	37.48
5.301 (0)	2.0 (2)	2.75 (0)	7.0 (0)	115.76
5.301 (0)	0 (-2)	2.75 (0)	7.0 (0)	65.38
5.301 (0)	1.0 (0)	4.00 (2)	7.0 (0)	54.34
5.301 (0)	1.0 (0)	1.50 (-2)	7.0 (0)	63.89
5.301 (0)	1.0 (0)	2.75 (0)	10.0 (2)	54.96
5.301 (0)	1.0 (0)	2.75 (0)	4.0 (-2)	40.72
5.301 (0)	1.0 (0)	2.75 (0)	7.0 (0)	54.12
5.301 (0)	1.0 (0)	2.75 (0)	7.0 (0)	60.02
5.301 (0)	1.0 (0)	2.75 (0)	7.0 (0)	91.34
5.301 (0)	1.0 (0)	2.75 (0)	7.0 (0)	55.67
5.301 (0)	1.0 (0)	2.75 (0)	7.0 (0)	61.94
5.301 (0)	1.0 (0)	2.75 (0)	7.0 (0)	50.92
5.301 (0)	1.0 (0)	2.75 (0)	7.0 (0)	49.34

[a] Vanillic acid concentration (mg L^{-1})
[b] Uncoded levels of factors
[c] (−2), (−1), (0), (1) and (2) are coded levels of factors

Sample preparation

Pineapple cannery waste consisting of peels and core were oven-dried in a Memmert Universal Oven at 40ºC for 7 days and ground in a Waring blender to pass a 4.75-mm sieve (Lee and Hanapi, 1997). The dried sample was kept at 4ºC until used.

Optimization of A. niger fermentation using RSM

The first step in the fermentation process involved the use of *A. niger* I-1472 for biotransformation of ferulic acid in pineapple cannery waste to vanillic acid. The cultivation experiments were conducted in 250-mL Erlenmeyer flasks containing 100-mL optimization media (Table 1). The medium described by Gross *et al.* (1990) was modified to contain (gL^{-1}): 20, maltose; 0.2, KH_2PO_4, 0.0132 $CaCl_2.2H_2O$; 0.5, $MgSO_4.7H_2O$; 0.0025, thiamine hydrochloride and 2 g dried pineapple waste. The amounts of diammonium tartrate and yeast extract added were varied according to the requirement of each fermentation experiment as shown in the Central Composite Rotatable Design in Table 1. Initial pH of each fermentation experiment was adjusted to the required value with HCl 0.5M. The optimization media were autoclaved for 15 min at 121°C. After autoclaving, *A. niger* I-1472 was inoculated into the optimization media which were then incubated at 30°C in an environmental shaker (Innova 4200 Incubator Shaker, New Brunswick Scientific) at 120 rpm for 7 days. At the end of A.

niger fermentation, solutions of the optimization media were centrifuged at 4000 rpm for 30 min. The supernatant was collected and 2 mL samples were filtered through nylon membrane filters (13 mm diameter, 0.45 µm pore size) (Millipore, USA) for High Performance Liquid Chromatography (HPLC) analysis of ferulic acid and vanillic acid. The remainder of the supernatant was used for *P. cinnabarinus* fermentation for vanillin production.

Biotransformation of vanillic acid by P. cinnabarinus

The second step involved the biotransformation of vanillic acid produced from *A. niger* fermentation to vanillin using *P. cinnabarinus* MUCL 39533. Five-mL spore suspensions of *P. cinnabarinus* were used in these experiments which were conducted using 60 mL optimum-point medium as determined using RSM in 250-mL Erlenmeyer flasks agitated at 120 rpm in an environmental shaker (Innova 4200 Incubator Shaker, New Brunswick Scientific) at 30°C for 7 days. The optimum-point medium formulation obtained from RSM experiments were used (n = 2) and the effect of Amberlite XAD-4 resin on vanillin production was tested. The resin was added on day-4 of fermentation to adsorb vanillin produced during fermentation.

Regeneration and elution of Resin XAD-4

Before use, the resin was washed in methanol to remove impurities and subsequently with distilled water to remove the methanol. For each experiment, 5 gL^{-1} resin was used. The resin was placed into cotton cloth pouches measuring 4 cm by 4 cm, submerged in distilled water in a beaker and autoclaved at 121°C for 15 min before use.

After 7 days of fermentation, vanillin trapped in the resin was eluted by using ethyl acetate solvent (1:2, w/v) as described by Hua *et al.* (2007) with modifications, whereby, ethyl acetate was used instead of butyl acetate. The resin and ethyl acetate mixture were shaken at 200 rpm in an environmental shaker (Innova 4200 Incubator Shaker, New Brunswick Scientific) at 30ºC for 2 h. The vanillin extracted was concentrated with the use of a rotary evaporator at 60°C (Buchi Rotavapor R-114). The concentrated vanillin was subsequently diluted with HPLC grade methanol for HPLC analysis.

Analytical methods

Ferulic acid extraction from dried pineapple waste materials

Ferulic acid extraction was performed on autoclaved and non-autoclaved pineapple waste powder as described by Tilay *et al.* (2008) with slight modifications followed by Solid Phase Extraction. Approximately 20 g of pineapple powder was autoclaved at 121°C for 15 min. Two gram of autoclaved and non-autoclaved pineapple powder was placed in a 250-mL Erlenmeyer flask with 60 mL NaOH 2M and shaken in an environmental shaker for 24 h at room temperature. Sodium hydrogen sulfite (0.001 g) was then added to the solution to prevent degradation of ferulic acid. The alkaline hydrolysate was centrifuged at 4000 rpm for 15 min. The pH of the resulting supernatant was adjusted to pH 1.8 with 2M HCl. Ferulic acid was extracted from the supernatant by shaking thrice with ethyl acetate (60 mL) in a separatory funnel. The organic layer was collected and concentrated to approximately 2 – 3 mL in a Buchi Rotavapor R-114 at 60ºC. Two mL of the concentrate was then dissolved in 2 mL of acetonitrile:water (1:1). The extract was then treated using Solid Phase Extraction (Tian *et al.*, 2005). A Sep-Pak® Vac 6 mL C18 cartridge (Waters, Milford, MA, USA) which had previously been activated with methanol (6 mL) and standardized with 12 mL acidified water containing 0.025% trifluoroacetic acid (TFA) to achieve pH 2.45. Prior to use, the cartridge was washed with 6 mL acidic water : methanol (9 : 1, v/v). Two mL of the extract was eluted with 3 mL acidic water : methanol (3 : 7, v/v) at a rate of 1 – 2 mL min^{-1}. The first 1.5 mL eluted was discarded, the subsequent 1.5 mL of elution was collected and added with distilled water to 2 mL in volume for HPLC analysis.

Ferulic acid, vanillic acid and vanillin determination by HPLC

The HPLC system consisted of a C18 column (Waters Symmetry® C18 5 µm, 4.6 x 150 mm) and UV/VIS detector (Waters 484 variable wavelength HPLC UV-VIS) set at 254 nm. An isocratic elution mode with aqueous 1 mM trifluoroacetic acid: methanol (17:8) degassed mobile phase was used at flow rate 1.0 ml/min flow rate controlled by a Waters 484 Variable Wavelength HPLC UV-VIS pump (Sachan *et al.*, 2004).

Experimental design and statistical analysis

A central composite rotatable design (CCRD) was used to allocate treatment combinations (Table 1). Four factors were studied to optimize *A. niger* fermentation to produce vanillic acid, i.e., inoculum size, yeast extract (Merck, Germany) concentration, diammonium tartrate (Merck, Germany) concentration and initial pH of the optimization

medium. The amount of vanillic acid produced from the fermentation process was assumed to be under the influence of these factors. To set up a statistical model, we let Y denote vanillic acid concentration (mgL^{-1}) while X_1, X_2, X_3 and X_4 denote inoculum size (log CFU mL^{-1}), yeast extract (gL^{-1}), diammonium tartrate (gL^{-1}) and initial pH of the optimization medium, respectively. The actual factor levels corresponding to the coded factor levels are shown in Table 1. In our regression model, the response variable was vanillic acid produced and candidates for explanatory variables were linear, interaction and quadratic terms of coded levels of inoculum size, yeast extract, diammonium tartrate and initial pH of the optimization medium. Data were analyzed by using Design-Expert® version 6.0.10 for Windows software. The level at which every term in the selected model should be significant was set as 5%.

Results and Discussion

Ferulic acid content determination

Ferulic acid extraction was more effective in pineapple powder which had undergone autoclaving (3.25 ± 0.12 mgg^{-1}) than non-autoclaved pineapple powder (0.64 ± 0.03 mgg^{-1}). The simple thermal pretreatment method employed in this study was able to improve solubilisation of ferulic acid ester-linked to the cell wall components. Similar improvement of ferulic acid solubilisation resulting from thermal pretreatment was also reported by Saulnier *et al.* (2001) in their work on maize bran. Thus in this study, pineapple powder used for *A. niger* fermentation was autoclaved before use in order to facilitate microbial feruloyl esterases action during fermentation.

Optimizing vanillic acid production from A. niger fermentation of pineapple waste

Developing a regression model

A five-level, four-factor CCRD was used to estimate the relationship between the factors and production of vanillic acid. The CCRD consisted of 16 factorial points, 8 axial points and 7 center points. The experiments were run in random order to minimize the effects of unexpected variability in the responses due to extraneous factors. Table 1 shows the treatment combinations in the CCRD and their respective responses. The experimental data was fitted to a second-order polynomial model where the response variable was vanillic acid concentration and the candidates for explanatory variables were linear, interaction and quadratic terms of *A. niger* inoculum size, yeast extract, diammonium tartrate and pH. This model was significant ($P < 0.05$) with $r^2 = 0.8098$ and coefficient of variation = 20.50% (Table 2). Its lack of fit was insignificant ($P > 0.05$).

Table 2. Analysis of variance of the second order regression model[a]

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value	P value
Model	14	13761.91	982.99	4.86	0.0017
Residual	16	3232.90	202.06		
Lack of fit	10	1999.14	199.91	0.97	0.5395
Pure error	6	1233.76	205.63		
Total	30	16996.19			

[a] $r^2 = 0.8098$, coefficient of variation = 20.50%

Table 3. Coefficient estimates in the regression model

Variable	Coefficient estimate	Standard error	P value	t value
Intercept	$b_0 = 60.48$	5.37	0.0017	11.26
X_1	$b_1 = 5.11$	2.90	0.0972	1.76
X_2	$b_2 = 9.39$	2.90	0.0052	3.24
X_3	$b_3 = -3.92$	2.90	0.1952	-1.35
X_4	$b_4 = -3.85$	2.90	0.2027	-1.33
X_1X_2	$b_{12} = 6.41$	3.55	0.0904	1.80
X_1X_3	$b_{13} = -5.24$	3.55	0.1594	-1.48
X_1X_4	$b_{14} = -5.28$	3.55	0.1567	-1.49
X_2X_3	$b_{23} = -16.07$	3.55	0.0003	-4.52
X_2X_4	$b_{24} = -0.010$	3.55	0.9978	0
X_3X_4	$b_{34} = -10.89$	3.55	0.0074	-3.06
X_1^2	$b_{11} = 1.83$	2.66	0.5009	0.69
X_2^2	$b_{22} = 9.39$	2.66	0.0028	3.53
X_3^2	$b_{33} = 1.52$	2.66	0.5749	0.57
X_4^2	$b_{44} = -1.30$	2.66	0.6323	-0.49

The functional form of this model was as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2$$

(Equation 1)

Table 3 shows how the above model was fitted to the data. Based on the significance of the model, insignificance of its lack of fit, r^2 and coefficient of variance values, this model was considered suitable and capable of representing data in the experimental region.

Finding the optimum point of the factors

Optimization of the fermentation was conducted by calculating and searching for the maximum response using the Design-Expert® version 6.0.10 for Windows software. The optimum point obtained was (X_1, X_2, X_3, X_4) = (0.498, -1.641, 1.952, -1.99). These coded levels were uncoded to obtain the following results: $X_1 = 3.55 \times 10^5$ CFU mL^{-1}, X_2 = 0.18 gL^{-1} yeast extract, $X_3 = 3.97$ gL^{-1} diammonium tartrate and X_4 = pH 4.02. The estimated response under these optimum conditions was 162.55 mgL^{-1} vanillic acid. A validation test was conducted under these optimum conditions to confirm the optimum response value predicted by the model. Vanillic acid

Table 4. Vanillin production from vanillic acid by *P. cinnabarinus* MUCL 39533 (n = 2)

Medium condition	Vanillin (mg L^{-1})
Optimum-point medium	36.50
Optimum-point medium + resin	141.00

produced in the validation test was 173.44 ± 7.52 mgL^{-1}. The difference between the calculated and experimental response was merely 6.43%, indicating that the model was capable of representing the data in the experimental region well.

The t-value, coefficient estimate and P-value are commonly used as partial or marginal tests for testing the significance and value of regressor variables in a regression model (Myers *et al*., 2009). It was observed that the variables X_2, X_2^2 and X_2X_3 had higher t-values and coefficient estimates, and lower P-values, than the other variables present in the model. This indicated that X_2 (yeast extract) played a strong role in vanillic acid production by *A. niger* fermentation of pineapple cannery waste. Nitrogen and carbon sources are essential components of fermentation media to support biomass production, cell activities and metabolite formation (Zhang *et al*., 2003). In addition, major elements such as carbon, hydrogen, oxygen and nitrogen, minor elements, namely, phosphate, potassium, sulfate, and magnesium, vitamins and hormones are also required or desirable. Yeast extract contains peptides and free amino acids in addition to essential growth factors such as minerals, vitamins and other metabolites, which may explain their strong influence on microbial fermentation as shown in this study.

Biotransformation vanillic acid to vanillin by P. cinnabarinus MUCL 39533

The use of Amberlite XAD-4 resin significantly improved vanillin yield (Table 4). This nonionic polymeric resin composed of polystyrene chains cross-linked with divinylbenzene, was used for adsorption of vanillin and to reduce subsequent transformation of vanillin to vanillyl alcohol or vanillic acid. Low vanillin yield from ferulic acid has frequently been reported due to the high rate of vanillin degradation (Hua *et al*., 2007). In fermentation processes involving products which are toxic to the producing organisms, as is the case with vanillin, in-situ product removal techniques using adsorbent resin can be applied (Freeman *et al*., 1993), the positive effect of which is clearly seen in this study.

In this study, *A. niger* I-1472 which is capable of producing polysaccharide-degrading enzymes including feruloyl esterases has enabled the release of ferulic acid from pre-thermal treated pineapple cannery waste, a cheap and easily obtained agricultural byproduct. This agro-waste material was used directly after autoclaving without the need for further complicated pretreatment. The vanillic acid produced from *A. niger* fermentation was then biotransformed into vanillin by *P. cinnabarinus* in the presence of XAD-2 resin. By using RSM, the optimum conditions for vanillic acid production by *A. niger* was determined. The biotransformation of vanillic acid to vanillin production was then further improved by the use of Amberlite XAD-4 resin in the medium for *P. cinnabarinus* fermentation to adsorb vanilin and prevent its deterioration to vanillyl alcohol and vanillic acid.

Conclusion

The optimum conditions for vanillic acid and vanillin production from pineapple cannery waste were established by using RSM. The estimated conditions for optimal vanillic acid production was inoculum size, 3.08×10^5 CFU mL^{-1}; yeast extract, 0.37 gL^{-1}; diammonium tartrate, 3.88 gL^{-1} and initial pH, 4.3. Subsequent biotransformation of vanillic acid by *P. cinnabarinus* MUCL 39533 to vanillin was enhanced with the addition of Amberlite XAD-4 resin.

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References

Freeman, A., Woodley, J. M. and Lilly, M. D. 1993. *In-situ* product removal as a tool for bioprocessing. Nature Biotechnology 11:1007 – 1012.

FruiTrop. 2007. Dossier du mois l'ananas 144:6.

FruiTrop. 2008. Dossier du mois: l'ananas 154:6 – 30.

Gross, B., Yonnet, G., Picque, D., Bruneire, P., Corrieu, G. and Asther, M. 1990. Production of methylthranilate by the basidiomycete *Pycnoporus cinnabarinus*. Applied Microbiology and Biotechnology 34:387-392.

Hua, D. L., Ma, C. Q., Song, L. F., Lin, S., Zhang, Z. B., Deng, Z. X. and Xu, P. 2007. Enhanced vanillin production from ferulic acid using adsorbent resin. Applied Microbiology and Biotechnology 74:783-790.

Lee, T. H. and Hanapi, M. 1997. The potential of citric acid production using pineapple waste. Malaysia, Johor: Universiti Teknologi Malaysia, MSc thesis

Lesage-Meessen, L., Delattre, M., Haon, M., Thibault, J.-F., Ceccaldi, B. C. and Brunerie, P. 1996. A two-step bioconversion process for vanillin production from ferulic acid combining *Aspergillus niger* and *Pycnoporus cinnabarinus*. Jurnal of Biotechnology

50:107–113.

Internet: Malaysian Pineapple Industry Board. 2011. Pineapple Industry Information. Johor, Malaysia. Downloaded from *http://www.mpib.gov.my/c/document_ library/get_file?uuid=1ed1a5bd-50a0-4242-97482c37610f879b&group Id=22005.* on 24/5/2012.

Myers, R. H., Montgomery, D. C. and Anderson-Cook, C.M. 2009. Response Surface Methodology: Process and product optimizat44ion using designed experiments. New Jersey: John Wiley & Sons, Inc.

Priefert, H., Rabenhorst, J. and Steinbuchel, A. 2001. Biotechnology production of vanillin. Applied Microbiology and Biotechnology 56:296-314.

Sachan, A., Ghosh, S. and Mitra, A. 2004. An efficient isocratic separation of hydroxycinnamates and their corresponding benzoates from microbial and plant source by HPLC. Biotechnology and Applied Biochemistry 40:197-200.

Saulnier, L., Marot, C., Elgorriaga, M., Bonnin, E. and Thibault, J.-F. 2001. Thermal and enzymatic treatments for the release of free ferulic acid from maize bran. Carbohydrate Polymers 45:269-275.

Stentelaire, C., Lesage-Meessen, L., Oddou, J., Bernard, O., Bastin, G., Ceccaldi, B. C. and Asther, M. 2000. Design of a fungal bioprocess for vanillin production from vanillic acid at scalable level by Pycnoporus cinnabarinus. Journal of Bioscience and Bioengineering 89 (3):223-230

Tian, S., Nakamura, K., Cui, T. and Kayahara, H. 2005. High performance liquid chromatographic determination of phenolic compounds in rice. Journal of Chromatography A 1063:121-128.

Tilay, A., Bule, M., Kishenkumar, J. and Annapure, U. 2008. Preparation of ferulic acid from agricultural wastes: Its improved extraction and purification. Journal of Agricultural and Food Chemistry 56 (17):7644–7648.

Zhang, J., Reddy, J., Buckland, B. and Greasham, R. 2003. Towards consistent and productive complex medium for industrial fermentations: Studies on yeast extract for a recombinant yeast fermentation process. Biotechnology and Bioengineering 82(6):640-652.