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Production of Resveratrol by Piceid Deglycosylation Using Cellulase

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Abstract: Resveratrol is a dietary polyphenolic compound widely used in medicine, food, and cosmetic products. The glycoside form of resveratrol, piceid, is also present in several plant materials but is less bioavailable. In this study, enzymatic transformation of piceid into resveratrol using inexpensive cellulase was investigated. Response surface methodology was used to evaluate the effect of reaction parameters, including reaction temperature, reaction time, enzyme amount and pH. The optimal conditions for biotransformation of piceid to resveratrol are: a reaction temperature of 50 °C, reaction time of 4.75 h, enzyme amount of 2.5 fungal β-glucanase (FBG) units and pH of 4.3. In addition, the extracts from *Polygonum cuspidatum* root contained high amounts of piceid were treated with cellulase in order to deglycosylation that increased resveratrol yield. After treatment, the resveratrol yield significantly increased from 2.72 to 9.49 mg/g, while the piceid contents decreased from 8.60 to 0 mg/g. The result provides an efficient method to convert piceid in the extracts of *P. cuspidatum* root into resveratrol by cellulase.

Keywords: cellulase; piceid; resveratrol; *Polygonum cuspidatum*; enzymatic transformation

1. Introduction

Natural polyphenolic compounds are usually secondary plant metabolites. Of them, phytoalexins are synthesized only in response to infection or injury and play an important role in the defense of plants against pathogens, parasites and UV radiation. Resveratrol (*trans*-3,5,4′-trihydroxystilbene) is a kind of phytoalexin belonging to the stilbene family [1]. It is present in natural foods and beverages (e.g., in grapes and red wine) and is widely consumed [2,3]. It has been suggested that moderate drinking of red wine helps to prevent cardiovascular disease; resveratrol in red wine is believed to be the key molecule due to its inhibitory activities against platelet aggregation and oxidation of low-density lipoprotein (LDL) in blood [4]. Resveratrol is also recognized as a bioactive agent with potent anti-inflammatory, antioxidant, anti-proliferative, and cancer chemopreventive activities [5,6].

Resveratrol exists in a variety of vegetable foods, such as *Vaccinium* spp. (including blueberry, bilberry, and cranberry), peanut and huzhang (*Polygonum cuspidatum*) [7–9]. In these foods, a glycosylated derivative of resveratrol, piceid (3,5,4′-trihydroxystilbene-3-*O*-β-D-glucopyranoside), is also present in good quantity [10]. Piceid is the major derivative of resveratrol in many plants

and is usually present in much higher amounts than resveratrol itself. The amount of piceid has been reported to be 3–7 times higher than that of resveratrol in grape berry skins, hop cones, grape juices, *P. cuspidatum* roots and cocoa-containing products [11–13]. It should be kept in mind that glycosylation of piceid to the resveratrol 3,5-β-D-diglucoside form increased the water solubility 1700-fold as compared to resveratrol [14]. The modification of resveratrol to affect its bioactivity may be useful for specific applications. This implies that whatever method is chosen for resveratrol modification, site selectivity requires special attention [14–16]. However, the bioavailability of piceid is lower than that of resveratrol. Studies have shown that resveratrol is absorbed more easily and rapidly than piceid by human intestinal cells [17]. Henry *et al.* have reported that piceid cannot be absorbed by the transport system located in the apical membrane of human intestinal Caco-2 cells [18], probably because resveratrol has a higher affinity toward the lipid bilayer of the cellular membrane than piceid. In *in vitro* tests, resveratrol metabolites did not elevate endothelial nitric oxide synthase enzyme activity and endothelial NO release or affect intracellular reactive oxygen species levels [19]. In order to increase the production of resveratrol, it is important to develop a deglycosylation technique to transform piceid in plant or food materials into resveratrol.

Deglycosylation can be achieved by chemical or enzymatic methods. In general, the chemical method uses acid or alkali as the hydrolytic reagent and often requires high temperatures or long incubation times, which may lead to further degradation of the hydrolysate (containing resveratrol and/or piceid). The hydrolysis of *P. cuspidatum* root by H_2SO_4 solution failed due to the fact resveratrol was substantially destroyed during the process [20]. In contrast, enzymatic hydrolysis usually takes place at moderate pH and temperature and is an environmental-friendly process without generation of toxic or degraded products [21,22]. β-Glucosidase has been used for deglycosylation of isoflavone glucosides in soymilk into their aglycones [23] and for hydrolysis of resveratrol glucosides in wine in order to analyze the total wine resveratrol [24]. However, the high cost of β-glucosidase is the major concern of enzymatic transformation reactions for industrial applications. The cost of enzymes is currently major factor limiting the commercialization on the enzymatic deglycosylation of natural botanical active ingredients for industrial production [25]. *Aspergillus* species are efficient producers of cellulase [26,27]. Cellulase is a multi-component carbohydrase presenting *endo*-1,4-β-glucanase (EC 3.2.1.4), *exo*-1,4-β-glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) activities, which breaks down cellulose through synergistic action [28,29]. Currently, enzyme cost is also the major concern of biofuel production from cellulosic biomass [30,31]. Literature estimates for the cost contribution of enzymes to the production of lignocellulosic ethanol vary significantly from 0.1 to 0.4 USD per gallon [32]. With the development of biomass ethanol, the cellulase cost will be gradually reduced. In contrast, natural botanical active ingredients are usually sold at a high price so the enzyme cost will become acceptable if the cost is around 0.1~0.4 USD per gallon product. Compared to the high price of β-glucosidase on the market, cellulase is relatively inexpensive and could be used in the food industry to transform piceid into resveratrol or deglycosylation of nature botanical compounds from plant materials.

Response surface methodology (RSM) is a combination of mathematical and statistical techniques. It can be used for designing experiments, building models, evaluating the relative significance of the reaction variables, and determining the optimum conditions for the desired responses [33]. Optimization of enzyme loading and the operating condition can improve the yield of products and thus cut down the cost [34]. In this study, the enzymatic transformation of piceid to the aglycone form resveratrol using cellulase was investigated. Statistical experiment design and response surface methodology (RSM) analysis were employed to understand the relationship between the reaction variables (temperature, time, enzyme amount and pH) and the conversion. The optimal transformation condition was obtained and tested experimentally with extracts of *P. cuspidatum* root.

2. Results and Discussion

2.1. Model Fitting

The main objective of this work was to develop a statistical approach to understand the relationship between the variables and the response (conversion) of an enzymatic reaction, *i.e.*, hydrolysis of piceid to resveratrol by cellulase. Factors that may affect the reaction include enzyme loading and reaction conditions (e.g., temperature, time, pH). In order to obtain a high conversion yield and reaction rate, optimization of the process is required [35]. In comparison with the one-factor-at-a-time design, RSM, as employed in this study, is more efficient in reducing the experimental runs and time for determination of the optimal condition. The experimental conditions and the response values of the experimental design are listed in Table 1. The highest conversion (98.39% ± 0.33%) was obtained at 55 °C for 6 h with enzyme amount 1.75 FBG and pH 4 (run No. 11), and the lowest conversion (0.37% ± 0.04%) was obtained at 55 °C for 3.5 h with enzyme amount 1.75 FBG and pH 2 (run No. 27). The manipulated variables and the responses were analyzed to fit a regression model, and the second-order polynomial model is as follows:

$$\begin{gathered} Y(\%) = -818.7237 + 17.2035X_1 + 47.0162X_2 + 73.6631X_3 + 130.1544X_4 - 0.15685X_1^2 - \\ 0.42367X_1X_2 - 0.58499X_1X_3 + 0.33325X_1X_4 - 3.38922{X_2}^2 + 1.65982X_2X_3 + 1.55617X_2X_4 \\ - 10.9288{X_3}^2 + 1.95963X_3X_4 - 18.359{X_4}^2 \end{gathered} \quad (1)$$

where Y is the predicted conversion; X_1 is the temperature; X_2 is the time; X_3 is the enzyme amount; X_4 is the pH.

Table 1. Central composite design and observed experimental data for 5-level-4-factor response surface analysis.

Run	Independent Variable				Conversion(%)
	X_1 (°C) Temperature	X_2 (h) Time	X_3 (FBG) Enzyme Amount	X_4 *pH*	Actual Values [a]
1	45	4.75	2.5	3	68.31 ± 3.71
2	35	3.5	1.75	4	39.04 ± 3.18
3	55	3.5	1.75	6	19.01 ± 0.04
4	55	3.5	1.75	4	80.91 ± 1.95
5	65	4.75	2.5	5	49.04 ± 7.55
6	45	2.25	2.5	5	52.25 ± 5.41
7	65	2.25	2.5	5	39.88 ± 1.78
8	45	2.25	1	5	30.34 ± 2.03
9	55	3.5	1.75	4	82.26 ± 2.70
10	45	4.75	1	3	27.13 ± 0.17
11	55	6	1.75	4	98.39 ± 0.33
12	55	1	1.75	4	25.49 ± 0.48
13	65	2.25	2.5	3	2.17 ± 0.09
14	65	4.75	1	3	1.23 ± 0.04
15	65	4.75	2.5	3	2.50 ± 0.18
16	65	2.25	1	5	20.03 ± 0.80
17	55	3.5	1.75	4	75.24 ± 1.64
18	45	4.75	2.5	5	84.21 ± 2.66
19	55	3.5	0.25	4	19.84 ± 1.25
20	55	3.5	3.25	4	97.24 ± 1.01
21	65	2.25	1	3	1.01 ± 0.08
22	65	4.75	1	5	31.83 ± 2.20
23	45	2.25	1	3	14.23 ± 0.21
24	45	4.75	1	5	56.85 ± 0.75
25	45	2.25	2.5	3	33.44 ± 1.33
26	75	3.5	1.75	4	1.73 ± 0.10
27	55	3.5	1.75	2	0.37 ± 0.04

[a] Mean of duplicate determinations.

Analysis of variance (ANOVA) was performed for the fitness of the model to the experimental data. The significance of each coefficient was determined by p-value and $p < 0.05$ indicates that the model term affects the hydrolytic process significantly. As shown in Table 2, the statistical analysis demonstrated that all of the four linear model terms, *i.e.*, X_1 (temperature), X_2 (time), X_3 (enzyme amount) and X_4 (pH), affected the process significantly ($p < 0.05$). The quadratic terms of ${X_1}^2$, ${X_2}^2$, and ${X_4}^2$ also affected the process significantly, of which ${X_1}^2$ and ${X_4}^2$ affected the process most significantly ($p = 0.0001$ and <0.0001, respectively). In contrast, all of the other interaction terms (X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4) did not affect the process significantly ($p > 0.05$). The coefficient of determination (R^2) of this model was 0.92, indicating excellent correlation between the independent variables. The p-value for lack of fit was 0.0669, indicating statistically insignificant lack of fit. The analysis of variance (ANOVA) indicated that the second-order polynomial model was an adequate representation of the actual relationship between the response and the significant variables.

Table 2. ANOVA for response surface models of all independent variables.

Factor [a]	Conversion (*Y*)			
	Sum of Squares	**df**	***F*-Value**	**Prob > *F***
Model	23,999.79	14	10.17	0.0001 *
Linear term				
X_1	3594.42	1	21.34	0.0006 *
X_2	3118.40	1	18.51	0.0010 *
X_3	3849.26	1	22.85	0.0004 *
X_4	2639.37	1	15.67	0.0019 *
Quadratic				
${X_1}^2$	5248.53	1	31.1	0.0001 *
${X_2}^2$	598.27	1	3.55	0.0839
${X_3}^2$	806.20	1	4.78	0.0492 *
${X_4}^2$	7190.4	1	42.69	<0.0001 *
Interactions				
X_1X_2	448.74	1	2.66	0.1285
X_1X_3	307.99	1	1.82	0.2012
X_1X_4	177.68	1	1.05	0.3246
X_2X_3	38.74	1	0.23	0.6401
X_2X_4	60.54	1	0.35	0.5599
X_3X_4	34.56	1	0.20	0.6586
Residual	2020.83	12	-	-
Lack of Fit	1993.05	10	14.34	0.0669
Pure Error	27.78	2	-	-
R-Squared	0.92	-	-	-

[a] Independent variables. X_1: Temperature, X_2: time, X_3: enzyme amount, X_4: pH. * Significant with $p < 0.05$.

2.2. Response Surface Analysis

The effects of reaction temperature ranging from 45 to 65 °C, reaction time ranging from 1 to 6 h and their mutual interaction on the yield with enzyme amount of 1.75 FBG and pH of 4 were further investigated. As shown in Figure 1a, a quadratic effect of both variables was observed, but reaction time had greater influence on the resveratrol yield than temperature. The increase in reaction time from 1 h to 6 h led to a curvilinear increase in yield from ~25% to ~90% at a given temperature between 45 °C and 50 °C and from ~20% to ~60% at a given temperature higher than 60 °C. This indicates that excessive heat energy inhibited enzyme activity by thermal denaturation. Takada *et al.* expressed β-glucosidase of *A. aculeatus* in *Saccharomyces cerevisiae* and found that while the optimal temperature of β-glucosidase activity was 50 °C, and the enzyme was stable below 50 °C [36]. Figure 1b shows

the effects of enzyme amount, reaction time and their mutual interaction on the resveratrol yield at reaction temperature of 55 °C and pH of 4. The yield increased with the increases of enzyme amount and reaction time. The resveratrol yield gradually leveled off at a higher enzyme amount, suggesting that sufficient amount of enzyme is achieved for transformation of piceid to resveratrol in this system. Since an excessive amount of enzyme did not effectively increase the yield, the amount of enzyme should be minimized in order to save cost. A reaction with reaction time of 6 h, enzyme amount of 2.75 FBG, reaction temperature of 55 °C and pH of 4 led to 90% yield (Figure 1b). Figure 1c shows the effects of pH, reaction time and their mutual interaction on the resveratrol yield and with enzyme amount of 1.75 FBG and reaction temperature of 55 °C. With this fixed enzyme amount and reaction temperature, pH affected enzyme activity significantly. With a given reaction time between 1 h and 6 h, the yield gradually increased to an optimum as the pH increased from 3 to 4.3, but decreased as the pH more than 4.3. This is consistent with the results from a previous study in which the optimal pH of β-glucosidase from *A. aculeatus* was between 4 and 5 [36].

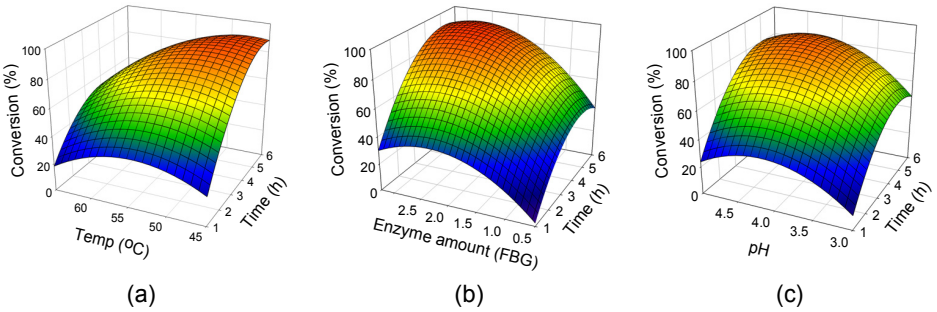

Figure 1. Response surface plot showing the relationships between resveratrol yield and reaction parameters: (**a**) reaction time and temperature; (**b**) reaction time and enzyme amount; and (**c**) reaction time and pH.

2.3. Attaining the Optimum Condition

The planned series of contour plots were generated from the predicted model (Equation (1)) by holding constant at a specific enzyme amount (1, 1.25, or 2.5 FBG) and pH 4, which are shown in Figure 2a–c, respectively. With this fixed pH, the yield increased with the increase in enzyme amount or reaction time, but the yield decreased with the increase in reaction temperature. The reaction time of 4.75 h, temperature of 50 °C, enzyme amount of 2.5 FBG, and pH of 4.3, (Figure 2c) possessed the highest predicted conversion with less reaction time required. Under these optimal conditions, the experimental results showed all the piceid was transformed into resveratrol.

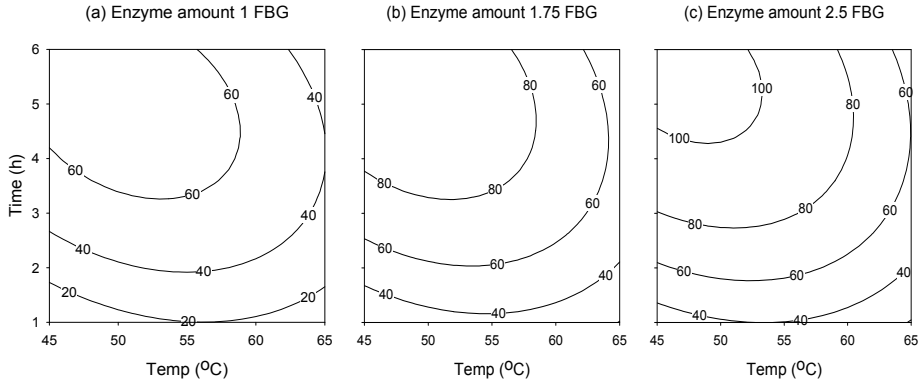

Figure 2. Contour plots of conversion of piceid to resveratrol with varying reaction temperature and times at constant enzyme amounts of (**a**) 1; (**b**) 1.75 and (**c**) 2.5 FBG. The numbers inside the contour plots indicate conversions under given reaction conditions.

2.4. Transformation of Piceid in the Extracts of P. cuspidatum Root

The optimum predicted conditions were tested with extracts of *P. cuspidatum* root which contained high amounts of piceid. A simple HPLC method was developed using a reversed phase C8 column with a gradient elution from 10% to 100% methanol. UV detection at 303 nm was used to enable detection of smaller amounts of piceid and resveratrol. Figure 3 shows two representative chromatograms of the extracts of *P. cuspidatum* root before and after cellulase treatment. It can be seen that the piceid peak is much higher than the resveratrol one in the extracts. However, the piceid was nearly transformed to resveratrol after cellulase treatment. As Figure 4 shows, the contents of piceid and resveratrol were determined to be 8.60 mg/g and 2.72 mg/g, respectively, in the extracts of *P. cuspidatum* root. After treatment with cellulase under the optimum conditions (reaction time of 4.75 h, temperature of 50 °C, enzyme amount of 2.5 FBG, and pH of 4.3), the contents of piceid and resveratrol in the extracts were 0 mg/g and 9.49 mg/g, respectively. All the piceid in the extracts was completely converted into resveratrol by cellulase under the optimum conditions. The resveratrol was increased by 3.5-fold in the extracts, but the conversion of piceid to resveratrol was only 79%. Probably, in the HPLC analysis of the extracts of *P. cuspidatum* root, the other compounds in the extracts influenced the quantification of the piceid or resveratrol concentrations. The *P. cuspidatum* fermented by *A. oryzae* during 24 h incubation converted piceid to resveratrol with the highest yield of resveratrol 13.5 mg/g, 3.6-fold higher than that obtained from microwave-assisted extraction [20]. However, the optimal enzyme activity temperature is much higher than that of microbial growth in the fermentation. Therefore, the RSM model may be useful to control the enzyme activities and optimize the fermentation to increase the yield of resveratrol. An experimental proof of the fact that the enzyme mixture extracted from the digestive tract of snails with the aim of converting piceid to resveratrol was first reported [37]. The composition of Viscozyme® L employed in this study was 59.9% water, 23% sucrose, 10% sodium chloride, 7% β-glucanase and 0.1% potassium sorbate. Viscozyme® L is produced according to FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemical Codex (FCC) with the recommended purity specifications for food-grade enzymes. In recent years, resveratrol has become widely available as a botanical dietary supplement in the United States. Therefore, the extracts of *P. cuspidatum* root treated with Viscozyme® L contained high amounts of resveratrol that can be directly used in commercial products.

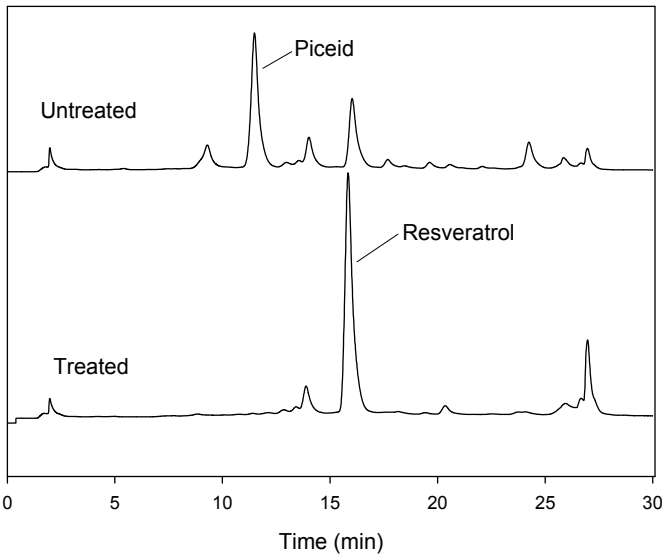

Figure 3. HPLC chromatogram of the extracts of *P. cuspidatum* root untreated and treated with cellulose.

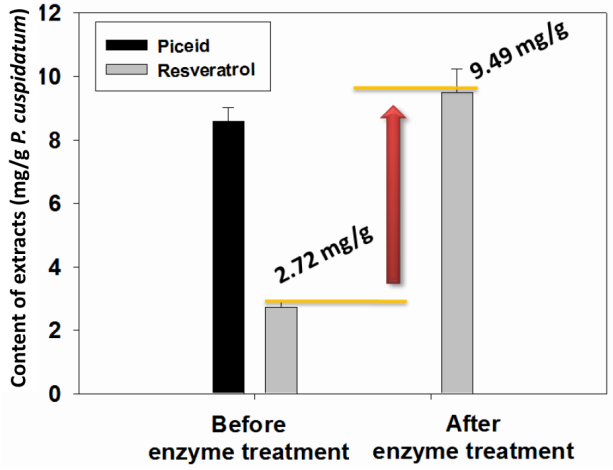

Figure 4. Cellulase-mediated transformation of piceid in the extracts of *P. cuspidatum* root into resveratrol under the optimum condition.

3. Experimental Section

3.1. Materials

Cellulase (Viscozyme® L (100 FBG/g), from *Aspergillus aculeatus* was purchased from Novozymes A/S (Bagsværd, Denmark). Piceid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Resveratrol was purchased from Changsha Nutramax Biotechnology (Changsha, China). Roots of *P. cuspidatum* were provided by Jing Jiue Co., Ltd. (Taichung, Taiwan). Unless otherwise specified, all reagents and chemicals used in this study were analytical grade.

3.2. Transformation of Piceid into Resveratrol

Piceid solutions (0.4 mg/mL) were prepared in 50 mM phosphate buffer at pH values ranging from 3 to 6 for use as substrate. Enzymatic transformation of piceid was performed in a glass tube containing 2 mg piceid and different amounts of cellulase (2.5–32.5 μL) in 5 mL 50 mM phosphate buffer. The glass tubes were placed in an orbital shaking bath (150 rpm) at different temperatures and for different reaction times. The reaction was terminated by addition of 5 mL ethanol to inactivate the cellulase. The solution was then centrifuged at 13,000 rpm for 10 min and the supernatant was withdrawn and analyzed by HPLC.

3.3. Quantitation of Piceid and Resveratrol by HPLC

Piceid and resveratrol were assayed by injecting 20 μL aliquot of the sample into a HPLC system (L-7400; Hitachi, Tokyo, Japan) using a ZORBAX Eclipse XDB C8 column (5 μM, 250 mm × 4.6 mm). Deionized water and methanol containing 0.1% acetic acid were used for gradient elution from 10% to 100% methanol for 20 min and then elution at 100% methanol for 5 min. The flow rate was set at 1.0 mL· min^{-1}. The UV detector was set at a wavelength of 303 nm. Calibration curves were prepared from piceid and resveratrol standards dissolved in water/ethanol (1:1). Piceid and resveratrol in samples were analyzed by comparing their retention times with those of the standards. The percent conversion of piceid to resveratrol was defined as increase of resveratrol per initial concentration of piceid × 100.

3.4. Experimental Design and Statistical Analysis

A five-level-four-factor central composite design including 27 experiments was employed in this study. To avoid bias, the 27 runs were performed in a random order. The variables and levels selected for the 27 runs were a reaction temperature of 35–75 °C, reaction time of 1–6 h, enzyme

amount of 0.25–3.25 FBG and pH of 2–6 (50 mM phosphate buffer, pH below 4 were adjusted by using 1 M HCl). Table 1 shows the uncoded independent factors (X_i), levels and experimental designs. Each experiment was carried out in duplicate. The software Design Expert (Trial Version 8.0.4, Stat-Ease Inc., Minneapolis, MN, USA) was employed for experimental design, data analysis and model building.

The data obtained from the central composite design was fitted into the quadratic polynomial model by regression analysis. The quadratic polynomial model for each response was as follows:

$$Y = \beta_{k0} + \sum_{i=1}^{4} \beta_{ki} X_i + \sum_{i=1}^{4} \beta_{kii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{kij} X_i X_j \quad (2)$$

where Y is the response (production rate); β_{k0}, β_{ki}, β_{kii} and β_{kij} are constant coefficients, X_i and X_j are the uncoded independent variables. The adequacy of the model was determined by evaluating the lack of fit, coefficient of regression (R^2) and the Fisher test value (F-value) obtained from the analysis of variance (ANOVA). Statistical significance of the model and model variables was determined at the 5% probability level ($p < 0.05$). Three-dimensional response surface plots were generated by keeping two of the four variables (reaction temperature, reaction time, enzyme amount or pH) at a fixed level and plotting that against remaining two variables.

3.5. Transformation of Piceid into Resveratrol in the Extracts of P. cuspidatum Root

Dried *P. cuspidatum* roots were ground into powder and passed through a 30 to 45 mesh sieve. The resulting powder was about 0.62 mm in size. One gram of the powder was extracted with 60 mL water in a sealed glass tube in a temperature-controlled ultrasonic bath (40 kHz, DC150H, Delta, New Taipei, Taiwan) set at 70 °C and 150 W of ultrasonic power for 30 min. After extraction, insoluble materials were removed by centrifugation at 13,000 rpm for 10 min, and 5 mL was used for the transformation reaction with cellulase under the optimal condition deduced by RSM.

4. Conclusions

Piceid could be transformed into resveratrol by commercial cellulase from *A. aculeatus*, and the enzyme amount, reaction temperature, reaction time, and pH affected the yield significantly. A 5-level-4-factor central composite design and RSM were employed for the experimental design and data analysis, and a regression model was obtained. The optimal transformation condition to obtain 100% yield was predicted to be an enzyme amount of 2.5 FBG, temperature of 50 °C, reaction time of 4.75 h, and pH of 4.3. Cellulase-mediated conversion of piceid into resveratrol under the optimum condition was tested using extracts of *P. cuspidatum* root. After the reaction, the resveratrol content in the extract increased from 2.72 mg/g to 9.49 mg/g, but the piceid content decreased from 8.60 mg/g to 0 mg/g. No piceid could be detected in the extracts after the reaction. The result provides a useful method to increase the production of resveratrol from plant materials. Cellulase is an inexpensive enzyme that has potential applications in the food industry for the large-scale transformation of piceid into resveratrol.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zernova, O.; Lygin, A.; Pawlowski, M.; Hill, C.; Hartman, G.; Widholm, J.; Lozovaya, V. Regulation of plant immunity through modulation of phytoalexin synthesis. *Molecules* **2014**, *19*, 7480–7496. [CrossRef] [PubMed]

2. Zhang, A.; Fang, Y.; Li, X.; Meng, J.; Wang, H.; Li, H.; Zhang, Z.; Guo, Z. Occurrence and estimation of *trans*-resveratrol in one-year-old canes from seven major chinese grape producing regions. *Molecules* **2011**, *16*, 2846–2861. [CrossRef] [PubMed]
3. Kurin, E.; Mučaji, P.; Nagy, M. *In vitro* antioxidant activities of three red wine polyphenols and their mixtures: An interaction study. *Molecules* **2012**, *17*, 14336–14348. [CrossRef] [PubMed]
4. Baur, J.A.; Sinclair, D.A. Therapeutic potential of resveratrol: The *in vivo* evidence. *Nat. Rev. Drug Discov.* **2006**, *5*, 493–506. [CrossRef] [PubMed]
5. Zhang, X.; Jiang, A.; Qi, B.; Ma, Z.; Xiong, Y.; Dou, J.; Wang, J. Resveratrol protects against helicobacter pylori-associated gastritis by combating oxidative stress. *Int. J. Mol. Sci.* **2015**, *16*, 26061–27769. [CrossRef] [PubMed]
6. Jang, M.; Cai, L.; Udeani, G.O.; Slowing, K.V.; Thomas, C.F.; Beecher, C.W.; Fong, H.H.; Farnsworth, N.R.; Kinghorn, A.D.; Mehta, R.G. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1997**, *275*, 218–220. [CrossRef] [PubMed]
7. Chen, B.Y.; Kuo, C.H.; Liu, Y.C.; Ye, L.Y.; Chen, J.H.; Shieh, C.J. Ultrasonic-assisted extraction of the botanical dietary supplement resveratrol and other constituents of *Polygonum cuspidatum*. *J. Nat. Prod.* **2012**, *75*, 1810–1813. [CrossRef] [PubMed]
8. Spier, A.P.; Bavaresco, C.S.; Wyse, Â.T.; Carvalho, D.; Freitas Sarkis, J.J. Effects of resveratrol and purple grape juice on nucleotide hydrolysis by adult rat serum. *Food Chem.* **2007**, *103*, 565–571. [CrossRef]
9. Djoko, B.; Chiou, R.Y.Y.; Shee, J.J.; Liu, Y.W. Characterization of immunological activities of peanut stilbenoids, arachidin-1, piceatannol, and resveratrol on lipopolysaccharide-induced inflammation of raw 264.7 macrophages. *J. Agric. Food Chem.* **2007**, *55*, 2376–2383. [CrossRef] [PubMed]
10. Wu, C.F.; Yang, J.Y.; Wang, F.; Wang, X.X. Resveratrol: Botanical origin, pharmacological activity and applications. *Chin. J. Nat. Med.* **2013**, *11*, 1–15. [CrossRef]
11. Kuo, C.H.; Chen, B.Y.; Liu, Y.C.; Chang, C.M.; Deng, T.S.; Chen, J.H.; Shieh, C.J. Optimized ultrasound-assisted extraction of phenolic compounds from *Polygonum cuspidatum*. *Molecules* **2013**, *19*, 67–77. [CrossRef] [PubMed]
12. Counet, C.; Callemien, D.; Collin, S. Chocolate and cocoa: New sources of *trans*-resveratrol and *trans*-piceid. *Food Chem.* **2006**, *98*, 649–657. [CrossRef]
13. Wang, L.; Xu, M.; Liu, C.; Wang, J.; Xi, H.; Wu, B.; Loescher, W.; Duan, W.; Fan, P.; Li, S. Resveratrols in grape berry skins and leaves in *Vitis germplasm*. *PLoS ONE* **2013**, *8*, e61642. [CrossRef] [PubMed]
14. Lepak, A.; Gutmann, A.; Kulmer, S.T.; Nidetzky, B. Creating a water-soluble resveratrol-based antioxidant by site-selective enzymatic glucosylation. *ChemBioChem* **2015**, *16*, 1870–1874. [CrossRef] [PubMed]
15. Kuo, C.H.; Hsiao, F.W.; Dai, S.M.; Chang, C.M.J.; Lee, C.C.; Liu, Y.C.; Shieh, C.J. Lipase catalyzed acetylation of 3, 5, 4′-trihydroxystilbene: Optimization and kinetics study. *Bioprocess Biosyst. Eng.* **2012**, *35*, 1137–1145. [CrossRef] [PubMed]
16. Kuo, C.H.; Hsiao, F.W.; Chen, J.H.; Hsieh, C.W.; Liu, Y.C.; Shieh, C.J. Kinetic aspects of ultrasound-accelerated lipase catalyzed acetylation and optimal synthesis of 4′-acetoxyresveratrol. *Ultrason. Sonochem.* **2013**, *20*, 546–552. [CrossRef] [PubMed]
17. Signorelli, P.; Ghidoni, R. Resveratrol as an anticancer nutrient: Molecular basis, open questions and promises. *J. Nutr. Biochem.* **2005**, *16*, 449–466. [CrossRef] [PubMed]
18. Henry, C.; Vitrac, X.; Decendit, A.; Ennamany, R.; Krisa, S.; Mérillon, J.M. Cellular uptake and efflux of *trans*-piceid and its aglycone *trans*-resveratrol on the apical membrane of human intestinal caco-2 cells. *J. Agric. Food Chem.* **2005**, *53*, 798–803. [CrossRef] [PubMed]
19. Ladurner, A.; Schachner, D.; Schueller, K.; Pignitter, M.; Heiss, E.; Somoza, V.; Dirsch, V. Impact of *trans*-resveratrol-sulfates and -glucuronides on endothelial nitric oxide synthase activity, nitric oxide release and intracellular reactive oxygen species. *Molecules* **2014**, *19*, 16724–16736. [CrossRef] [PubMed]
20. Wang, H.; Liu, L.; Guo, Y.X.; Dong, Y.S.; Zhang, D.J.; Xiu, Z.L. Biotransformation of piceid in *Polygonum cuspidatum* to resveratrol by *Aspergillus oryzae*. *Appl. Microbiol. Biotechnol.* **2007**, *75*, 763–768. [CrossRef] [PubMed]
21. Kuo, C.H.; Lee, C.K. Enhanced enzymatic hydrolysis of sugarcane bagasse by *n*-methylmorpholine-*n*-oxide pretreatment. *Bioresour. Technol.* **2009**, *100*, 866–871. [CrossRef] [PubMed]

22. Shafiee, A.; Tsipouras, A.; Bouffard, A.; Onishi, J.C.; Guan, Z.; Motamedi, H. Enzymatic deglycosylation of enfumafungin, a triterpene glycoside natural product, and its chemically synthesized analogues. *J. Mol. Catal. B* **2001**, *16*, 27–32. [CrossRef]
23. Horii, K.; Adachi, T.; Matsuda, T.; Tanaka, T.; Sahara, H.; Shibasaki, S.; Ogino, C.; Hata, Y.; Ueda, M.; Kondo, A. Improvement of isoflavone aglycones production using β-glucosidase secretory produced in recombinant *Aspergillus oryzae*. *J. Mol. Catal. B* **2009**, *59*, 297–301. [CrossRef]
24. La Torre, G.L.; Laganà, G.; Bellocco, E.; Vilasi, F.; Salvo, F.; Dugo, G. Improvement on enzymatic hydrolysis of resveratrol glucosides in wine. *Food Chem.* **2004**, *85*, 259–266. [CrossRef]
25. Matsakas, L.; Christakopoulos, P. Ethanol production from enzymatically treated dried food waste using enzymes produced on-site. *Sustainability* **2015**, *7*, 1446–1458. [CrossRef]
26. Decker, C.H.; Visser, J.; Schreier, P. β-glucosidases from five black *Aspergillus* species: Study of their physico-chemical and biocatalytic properties. *J. Agric. Food Chem.* **2000**, *48*, 4929–4936. [CrossRef] [PubMed]
27. Wen, Z.; Liao, W.; Chen, S. Production of cellulase/β-glucosidase by the mixed fungi culture *Trichoderma reesei* and *Aspergillus phoenicis* on dairy manure. *Process Biochem.* **2005**, *40*, 3087–3094. [CrossRef]
28. Kuo, C.H.; Lee, C.K. Enhancement of enzymatic saccharification of cellulose by cellulose dissolution pretreatments. *Carbohydr. Polym.* **2009**, *77*, 41–46. [CrossRef]
29. Naika, G.S.; Kaul, P.; Prakash, V. Purification and characterization of a new endoglucanase from *Aspergillus aculeatus*. *J. Agric. Food Chem.* **2007**, *55*, 7566–7572. [CrossRef] [PubMed]
30. Humbird, D.; Davis, R.; Tao, L.; Kinchin, C.; Hsu, D.; Aden, A.; Schoen, P.; Lukas, J.; Olthof, B.; Worley, M. *Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover*; National Renewable Energy Laboratory (NREL): Golden, CO, USA, 2011.
31. Lau, M.W.; Bals, B.D.; Chundawat, S.P.; Jin, M.; Gunawan, C.; Balan, V.; Jones, A.D.; Dale, B.E. An integrated paradigm for cellulosic biorefineries: Utilization of lignocellulosic biomass as self-sufficient feedstocks for fuel, food precursors and saccharolytic enzyme production. *Energy Environ. Sci.* **2012**, *5*, 7100–7110. [CrossRef]
32. Klein-Marcuschamer, D.; Oleskowicz-Popiel, P.; Simmons, B.A.; Blanch, H.W. The challenge of enzyme cost in the production of lignocellulosic biofuels. *Biotechnol. Bioeng.* **2012**, *109*, 1083–1087. [CrossRef] [PubMed]
33. Kuo, C.H.; Chen, H.H.; Chen, J.H.; Liu, Y.C.; Shieh, C.J. High yield of wax ester synthesized from cetyl alcohol and octanoic acid by lipozyme rmim and novozym 435. *Int. J. Mol. Sci.* **2012**, *13*, 11694–11704. [CrossRef] [PubMed]
34. Taherzadeh, M.J.; Karimi, K. Enzymatic-based hydrolysis processes for ethanol. *BioResources* **2007**, *2*, 707–738.
35. Chen, J.; Liu, D.; Shi, B.; Wang, H.; Cheng, Y.; Zhang, W. Optimization of hydrolysis conditions for the production of glucomanno-oligosaccharides from konjac using β-mannanase by response surface methodology. *Carbohydr. Polym.* **2013**, *93*, 81–88. [CrossRef] [PubMed]
36. Takada, G.; Kawaguchi, T.; Sumitani, J.I.; Arai, M. Expression of *Aspergillus aculeatus* No. F-50 cellobiohydrolase I (cbh1) and Beta.-glucosidase 1 (bgll) genes by *Saccharomyces* cerevisiae. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1615–1618. [CrossRef] [PubMed]
37. Wang, Z.; Zhao, L.C.; Li, W.; Zhang, L.X.; Zhang, J.; Liang, J. Highly efficient biotransformation of polydatin to resveratrol by snailase hydrolysis using response surface methodology optimization. *Molecules* **2013**, *18*, 9717–9726. [CrossRef] [PubMed]