Response Surface Analysis on the Microwave Integrated-Rumen Based Extraction of Natural Vanillin from Cured Vanilla Pods

IOP Conf. Series: Journal of Physics: Conf. Series **1295** (2019) 012016 doi:10.1088/1742-6596/1295/1/012016

Response Surface Analysis on the Microwave Integrated-Rumen Based Extraction of Natural Vanillin from Cured Vanilla Pods

V Paramita and ME Yulianto

Department of Industrial and Technology, Diponegoro University

Email: vparamita@live.undip.ac.id

Abstract. This study investigated the optimization of enzymatically extraction of vanillin from cured vanilla pods. The novelty is an establishment of enzymatic-hydrolysis extraction method conducted with a microwave heating source. These enzymes are able to disrupt the shell wall of the pods in order to shift the equilibrium phase, and thus enhance the extraction rate and yield. The observed parameters were type of enzymes (protease and cellulase), temperatures (30, 40, 50 °C), mass of enzymes (0, 6, 12, 24 gr) and extraction time (0, 10, 20, 30, 40, 50, 60 min). The protease and cellulase were isolated from rumen liquid. The experimental and mathematical analysis of vanillin content and reducing sugar content were performed by using high performance liquid chromatography and response surface methodology, respectively. The acquired coefficients of determination for protease and cellulase addition were 0.6801 and 0.8064, respectively. These results indicated that the vanillin content obtained by cellulase addition provide a better model accuracy than that of the protease. In order to yield the predicted optimum value of vanillin content by cellulase addition at 118.15 mg/L, the attained critical parameters were 35 °C of temperature with 22.89 of mass of cellulase (or 1:26.2 of enzyme-substrate ratio) during 38.52 min.

1. Introduction

Vanilla flavor is extensively applied in many industries of food and beverages, perfumery and pharmaceutical. This natural flavor could be extracted from cured vanilla pods, which provided a complex mixture of more than hundreds components with vanillin (4-hydroxy-3-methoxybenzaldehyde, C8H8O3) as its main component [1–4]. The cured pods vanilla extract offers a smooth, delicate and soothing aroma, which is improbable provided by synthetic human technologies so far. The natural vanillin production occupied less than 1% annually in the worldwide market in regard to the fluctuating value of US$ 1,200 and 4,000 per kg, while the synthetic one remains with as highest price as US$ 15 per kg [5]. The expensive price of natural vanillin is proportionally supported by their premium taste and fragrance with minimum supply all over the world because of their complicated treatment process during on- and off-farm stages [3,6,7].

Mature vanilla pods did not provide any aromatic flavor, however it contains the precursor of vanillin. In this condition, the vanillin bound to sugar as a glycoside, namely glucovanillin ($C_{14}H_{18}O_8$). The hydrolysis of glucovanillin performed many compounds with favorable aroma develops by the curing process [3,8]. This curing process required enzymes in order to disrupt the shell wall of mature pods cellular compounds and serve the hydrolysis reaction of the vanillin precursor of glucovanillin by

interacting with β-glucosidase [7]. Vanillin extraction of natural-matured vanilla pods [1,2,9,10] or natural-cured vanillin [11,12] interested many researchers due to their premium quality, price and minimum supply over worldwide. Conventional methods of hydrolysis-extraction of vanillin beans employed high quantities of organic solvent with prolonged periods of time [10], however obtained a low extraction mass transfer rate. Conventional heating of extraction provided high risk of heat loss, while microwave-assisted extraction decreases the heat loss and time consumption and also provides higher extraction rates with a lesser requirement of chemicals [13]. Modified approach by applying microwave integrated-heating method and rumen liquid enzymes during treatment provided more effective process than the conventional one [14,15].

In order to increase the extraction rate and yield, many studied proposed the application of enzymes addition. Many investigators studied the use of various commercial enzymes such as pectinase and β-glucosidase on green vanilla pods [8] and cellulase on dried vanilla pods [12]. Nevertheless, the costly commercial enzymes cause ineffectiveness on the economic advisability for its mass scale production. Therefore, the alternative application of non-commercially enzyme promises good opportunity. Tea leaf enzyme extract was studied in order to minimize the costly commercial enzymes for extracting the vanillin from the dried pods [6]. Since rumen fluids contain the hydrolytic enzymes, such as cellulase, protease, pectinase [16], Paramita and Yulianto promoted the application of isolated enzymes from rumen liquid on the vanillin extraction process [2]. One of the most active polysaccharide-degrading enzymes was including cellulase, which produced by rumen liquid anaerobic fungi [17,18]. Moreover, the application of tea leaf enzymes for enhancing the vanilla flavor compounds already patented [11]. Currently, most researchers applied response surface methodology (RSM) to optimize their parameters process in order to reduce the number of their experimental runs for evaluating the multiple parameters and their interactions [9,19,20]. Regarding to our preliminary study using the rumen isolated of enzymatic technique, the heating time and temperature played the key factors for influencing the vanillin hydrolysis-extraction. This study investigated the effects of extraction time, temperature, enzyme type obtained from rumen liquid and enzyme quantity on vanillin extraction, and optimized the parameters condition using RSM.

2. Materials and Methods

2.1. *Materials*

Cured green vanilla pods were collected from Temanggung, Central Java, Indonesia. Rumen liquid was provided from the slaughterhouse from the sub-region area of Semarang, Indonesia. Water and methanol of high performance liquid chromatography (HPLC) grade were obtained from Merck (Darmstadt, Germany). Vanillin standard was obtained from Sigma-Aldrich (Darmstadt, Germany).

2.2. *Protease and Cellulase Isolation from Rumen Fluid*

Rumen fluid was isolated from bovine rumen by filtration under cold conditions (4 °C). The filtrate was centrifuged at 10,000 g for 10 min at 4 °C to separate the supernatant (as the source of crude enzyme) from the microbial cell contents. Then, this supernatant was treated by adding 60% ammonium sulfate to obtain protease [21] and 80% for cellulase [22]. The mixture was stirred using a magnetic stirrer for 1 hour and allowed for 24 hours at 4 °C. This treated supernatant was centrifuged again at 10,000 g for 15 min at 4 °C. Ammonium sulfate fraction with the highest specific activity occurred prior to fractionation of subjected enzyme (protease at 60% and cellulase at 80%). The precipitate compound of each enzyme treatment was separated from the mixture and dissolved it in phosphate buffer pH 7.0 with a ratio of 10:1 (100 ml of precipitated supernatant was dissolved in 10 ml of phosphate buffer pH 7.0) for following application [15].

2.3. *Microwave Integrated Enzymatic Extraction*

Six hundred grams of cured vanilla pods were blended. Six thousand milliliters of distilled water were placed in microwave integrated extractor type EM-02 (Teguh Jaya Teknik Corp., Ungaran, Indonesia)

and set on the operation condition of extraction (30, 40 and 50 °C). After the condition was achieved, the cut vanilla pods were added onto extractor. Then, the isolated protease from rumen fluid was added with ratio of enzyme to the substrate of 1:50 (12 gr) and 1:100 (6 gr), whereas, cellulase was added with ratio of 1:25 (24 gr) and 1:50 (12 gr). The mixture without enzyme was also studied to find the comparative data. Samples were taken at 0, 10, 20, 30, 40, 50 and 60 minute. All of the contents were kept in suspension by using an agitator. The amounts of vanillin were quantified by using HPLC.

2.4. *Vanillin content measurement*

The concentration of vanillin was analyzed by using High Performance Liquid Chromatography (HPLC) Alliance 2695 with Photodiode Array Detector 2996 (Waters Corp., MA, United State). One milliliter of samples was centrifuged for 10 min at 13,000 rpm and separated the filtrate. Ten microliters of sample was then injected into HPLC. The column applied was Symmetry C18, 5 μm, 150 mm × 4.6 mm with 1 mL/min of flow rate. The mobile phases were H2O and methanol with ratio of 60:40 % of water/methanol for 10 min. Standard curve was obtained using vanillin standard [2].

2.5. *Reducing sugar content measurement*

The reducing sugars released were determined by using the 3,5-dinitrosalicylic acid method [23]. It was analyzed by employing the UV-Visible recording spectrophotometer UV-160A (Shimadzu Corp., Kyoto, Japan) at 550 nm of wavelength.

2.6. *Response Surface Methodology (RSM)*

In order to execute RSM, the design of experiments (DoE) applied was central composite designs (CCD) with design characteristic of face centered star points (e.g., star points ± 1). Software Statistica 8.0 (StatSoft, Dell Software, Texas, United States) was used in this study. A second-order model can be constructed efficiently with this methodology [24].

$$Y = R_0 + \sum_{i=1}^{k} R_i X_i + \sum_{i=1}^{k} R_{ii} {X_i}^2 + \sum_{i=1,i<j}^{k-1} \sum_{j=2}^{k} R_{ij} X_i X_j + \varepsilon \quad (1)$$

where X_i, X_j are input variables that affect the response Y; R_o, R_i, R_{ii} and R_{ij} (i = 1-k, j = 1-k) are a known parameters, and ε is a random error. A second order model is designed so that the variance of Y is constant for all points equidistant from the center of the design.

$$X_i = \left(\frac{X_i - X_0}{\Delta X_i}\right) \quad (2)$$

where Xi is the value of the code, Xo is the actual value at the center point and ΔX_i is the value of the pace of change [25].

Table 1. Experimental levels values of the independent variables by applying face centred star points.

Parameters	Variables	Coded levels		
		-1.000	0.000	1.000
Type of enzyme (-)	p		Protease	
Temperature (°C)	X_{1p}	30	40	50
Mass of enzyme (gr)	X_{2p}	0	6	12
Time (min)	X_{3p}	10	30	50
Type of enzyme (-)	c		Cellulase	
Temperature (°C)	X_{1c}	30	40	50
Mass of enzyme (gr)	X_{2c}	0	12	24
Time (min)	X_{3c}	10	30	50

CCD is a first-order (2N) design augmented by additional center and axial points to allow estimation of the tuning parameters of a second-order model. The design involves 2N factorial points, 2N axial points and 1 central point (16 runs) for each types of enzyme. The dependent variable was vanillin content (Y_1) and reducing sugar content (Y_2), while the independent variables were extraction temperature (X_{1p}; X_{1c}), mass of enzyme (or ratio of enzyme-substrate, X_{2p}; X_{2c}) and extraction time (X_{3p}; X_{3c}) regarding to the enzyme type obtained from rumen liquid (protease and cellulase). The range of experimental levels values applied on RSM study is presented in Table 1.

2.7. *Statistical analysis*

The experimental results of the response surface design were analyzed using Portable Statistica 8 Software (StatSoft Inc., CA, USA) and the linear regression analysis was reconstructed using Microsoft Excel 2010 Software (Microsoft Corp., Washington, USA). The p values less than 0.5 were taken into consideration [26].

3. Results and Discussion

3.1. *Effect of Enzymatic Extraction Time, Temperature and Enzyme on the Vanillin and Reducing Sugar Contents*

The hydrolyzed glucovanillin into vanillin and reducing sugar contents were obtained by 30 experimental runs and estimated by employing different combinations parameters of enzyme type, temperature, mass of enzyme (or enzyme-substrate ratio) and extraction time. Table 2 presents the experimental and predicted data of vanillin content and reducing sugar content treated by protease and cellulase. The residual value is the difference between experimental and predicted data [25]. The minimum residual value of vanillin content (3.344 mg/L) was achieved at 40 °C for 30 min by treating with the addition of 12 gr of protease while minimum residual value of reducing sugar content (0.009 gr/L) was achieved at 50 °C for 30 min by 12 gr of cellulase addition. The obtained reducing sugar contents during vanillin extraction established the main character of the sweet delicate aroma and taste of natural vanillin, which would not be able to occur in a synthetic vanilla [12]. It is concern able from the Figure 1A and 1B that the addition of different types of enzyme affected the extracted vanillin content and reducing sugar content, respectively. Amongst the 30 experimental conducted, the concentration of vanillin dispersed in the range of 12.23–311.72 mg/L and 47.89–285.99 mg/L by treatment with protease and cellulase, respectively, while reducing sugar concentration obtained about 1.54–10.00 gr/L and 1.53–2.47 gr/L by protease and cellulase addition, successively. Protease addition during extraction provided a larger range of the extracted vanillin content than that of cellulase addition. Without enzymes addition, the vanillin concentration gained 1.27 mg/L at 30 °C for 10 min of extraction time and increased up to 194.44 mg/L during 50 min at the same temperature. Increasing the temperature into 50 °C during the first 10 min without enzymes addition increased the vanillin content up to 34.61 mg/L, whereas no longer vanillin can be extracted exceeds 52.32 mg/L at 50 °C. All of these extraction processes were applying microwave as the heating source. The data obtained without enzymes addition indicated that longer time at low temperature affected the vanillin extracted, which possibly provided by a better hydrolyzed process on the cell degradation. Although increment of the temperature increased the extracted vanillin content slightly, however longer extraction time did not increase the vanillin content.

The maximum value of protease addition was gained at 30 °C during 30 min with enzyme-substrate ratio of 1:100 and the minimum value was obtained at 50 °C during 10 min with enzyme-substrate ratio of 1:50. On the contrary, the minimum extracted vanillin content with cellulase addition was gained at the lower temperature (30 °C) with longer time (30 min) than protease addition at the same ratio of enzyme-substrate. Opposite conditions of the maximum value of extracted vanillin content by protease addition was also applied to the cellulase addition. It was extracted at higher temperature (50 °C) for shorter time (10 min) with higher enzyme-substrate ratio (1:25). This condition suggested that the protease–rumen liquid isolated gave better activity at lower temperature than at the higher ones. Protease

provided a higher activity (0.75 U/mL) than cellulase activity (0.23 U/mL) at ambient temperature (30 °C) [15]. Although, most of proteolytic enzymes were provided an optimum temperature at the range of 40 and 60 °C [27,28], the obtained low optimum temperature (30 °C) of protease in our previous work [15] was in agreement with the low optimum temperature (30 °C) of protease produced from bacterium growth in animal intestines, in this case was marine animal [29]. On the contrary, the application of cellulase was gained the maximum value of vanillin content at 50 °C, due to the degradation (hydrolysis) the pods cell wall. It is in agreement that the optimum temperature of celullase activity in *Nectria catalinensis* was ranged from 50-55 °C [30].

Waliszewski *et al.* studied the kinetics of vanillin and sugar liberation from cured vanilla beans at 30, 40 and 50 °C of extraction temperature during 26 h (1 day 2 hours) by applying commercial cellulolytic enzyme. They postulated that enzymatic pretreatment enriches the vanillin content in the extract [12].

3.2. *Fitting models*

Applying multiple regression analysis on the experimental data, the following second order polynomial equations were found to represent the vanillin content (Y_1) and the reducing sugar content (Y_2) treated by protease and cellulase on Equation (3), (4), (5), (6), respectively, as follows:

$$Y_{1p} = 157.511 - 63.113\,X_{1p} + 1.227\,X_{2p} + 44.342\,X_{3p} + 122.161\,{X_{1p}}^2 - 123.757\,{X_{2p}}^2 - 165.192\,{X_{3p}}^2 + 20.063\,X_{1p}X_{2p} + 1.420\,X_{1p}X_{3p} - 41.802\,X_{2p}X_{3p} \quad (3)$$

$$Y_{1c} = 98.641 - 6.939\,X_{1c} + 82.477\,X_{2c} - 0.962\,X_{3c} - 22.046\,{X_{1c}}^2 - 9.360\,{X_{2c}}^2 + 84.758\,{X_{3c}}^2 + 22.149\,X_{1c}X_{2c} - 84.551\,X_{1c}X_{3c} - 126.807\,X_{1c}X_{3c} \quad (4)$$

$$Y_{2p} = 1.197 + 3.017\,X_{1p} + 1.811\,X_{2p} - 0.337\,X_{3p} + 1.918\,{X_{1p}}^2 + 1.810\,{X_{2p}}^2 + 1.699\,{X_{3p}}^2 + 3.101\,X_{1p}X_{2p} - 1.191\,X_{1p}X_{3p} - 0.900\,X_{2p}X_{3p} \quad (5)$$

$$Y_{2c} = 1.773 + 0.370\,X_{1c} - 0.554\,X_{2c} + 0.426\,X_{3c} + 0.264\,{X_{1c}}^2 + 0.620\,{X_{2c}}^2 + 0.446\,{X_{3c}}^2 - 0.139\,X_{1c}X_{2c} - 0.130\,X_{1c}X_{3c} - 0.039\,X_{2c}X_{3c} \quad (6)$$

The result of the second order response surface model fitting in the form of Analysis of Variance (ANOVA) of dependence variables are presented in the supplementary data. The ANOVA of the regression model of vanillin content demonstrates the F models for protease and cellulase addition. By applying the Fisher's F-test, it was performed that the value of F model for cellulase addition (24.99) was higher than the value of F model for protease addition (12.12). The significance of each coefficient to the dependent variables was determined by p-values ($p < 0.05$). The values of standardized effects were presented as an absolute value. Therefore, the highest value indicated the highest influenced parameters on the experimental design [31]. The quadratic model for extraction time gave the highest influenced parameters for protease addition, while the mass of protease performed the lowest influenced parameters. For cellulase addition, the interaction of mass of enzyme and the extraction time performed the highest influenced, while extraction time plays the lowest influenced parameters.

The accuracy of the models was performed by fitting the actual to the predicted values and performing the coefficient of determination (R-square/R^2) as their linearly trend lines (Figure 1). In this study, the value of the coefficient of determination showed 0.6801 and 0.8064 for vanillin content with protease and cellulase addition, respectively. These result indicated that the vanillin extracted data obtained by the addition of cellulase provided a better accuracy of model than the addition of protease. On the contrary, the model accuracy of sugar reducing on protease addition provided higher value (0.9228) than cellulase addition (0.7115).

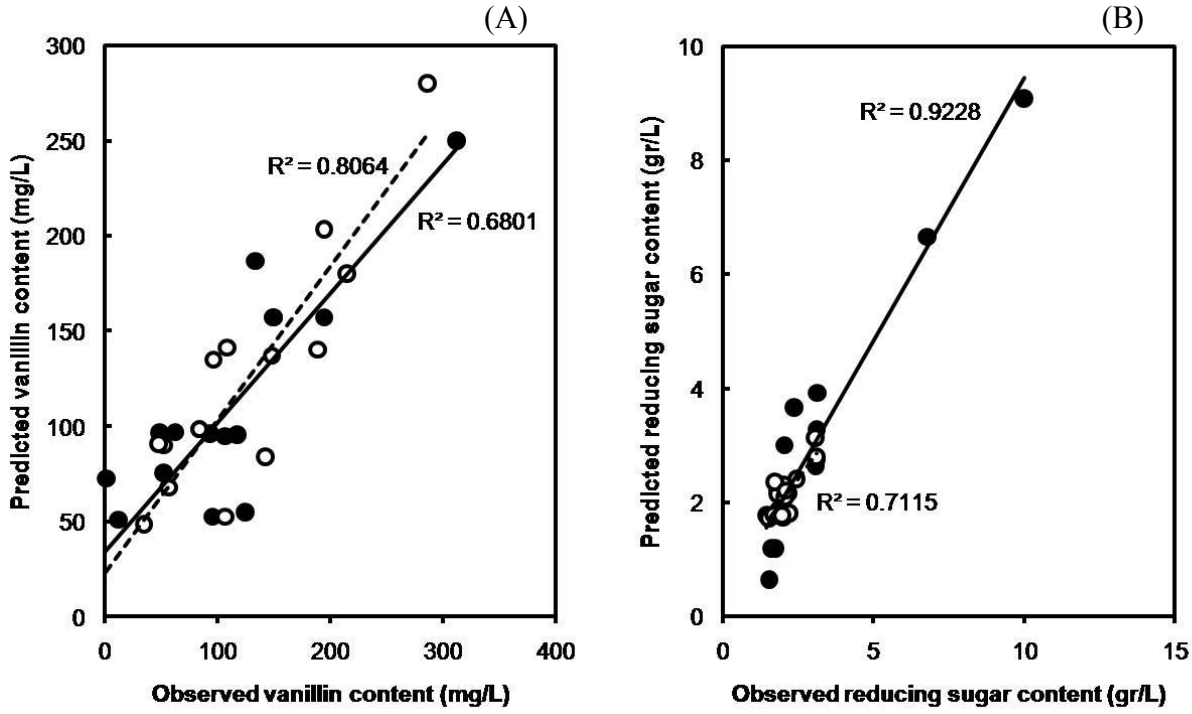

Figure 1. Observed and predicted values, obtained by applying CCD-face centred star points, of vanillin content (A) and reducing sugar content (B), treated by protease (●: observed versus predicted plots, —: trendlines) and cellulase (○:observed versus predicted plots, ---: trendlines).

3.3. *Response Surface Fitting on Observed Parameters*

Figure 2 represents the isoresponse contour and surface plots for the optimization of vanillin content which extracted by adding the protease. At constant extraction time of 50 min, the effects of the temperature and protease addition on the vanillin content was displayed in Figure 2A. The increasing of temperature and mass of protease decreased the vanillin content, generally. However, the higher vanillin content was observed at the temperature of 30 °C and 6 gr of protease addition. A similar effect on the response was observed for the extraction temperature at any level of time at 6 grams of protease addition (Figure 2B). An increase of temperature decreases the value of vanillin content while increasing the extraction time increased the vanillin content on their optimum level, following by the reverse trend. The higher vanillin content was observed at lower temperature of 30 °C on 30 minutes of extraction time. This condition was possibly provided because of the increasing temperature are able to damage the protease thus reducing the hydrolysis rate of cell wall in the vanilla pods and slow down the extraction rate of vanillin content. However, the further increasing temperature is able to distract the cell wall in the plants [32], regardless to the presence of the enzyme. This further cell wall damage was continuing the extraction process of vanillin content. Meanwhile, longer time at lower temperatures provided higher vanillin content than thus vice versa. This could be due to the minimum damage of protease with a relatively longer extraction time resulted a better degradation of cell walls by the enzyme presence [29].

The interaction effect of the protease addition and extraction time on the vanillin content at 40 °C in Figure 2C indicates a proper combination for vanillin extraction. An increase in the protease addition with time for extraction increased the vanillin content gradually but at a higher mass of protease and time the trend is reversed. The optimum for maximum vanillin content lies near the center point of the mass of protease and extraction time.

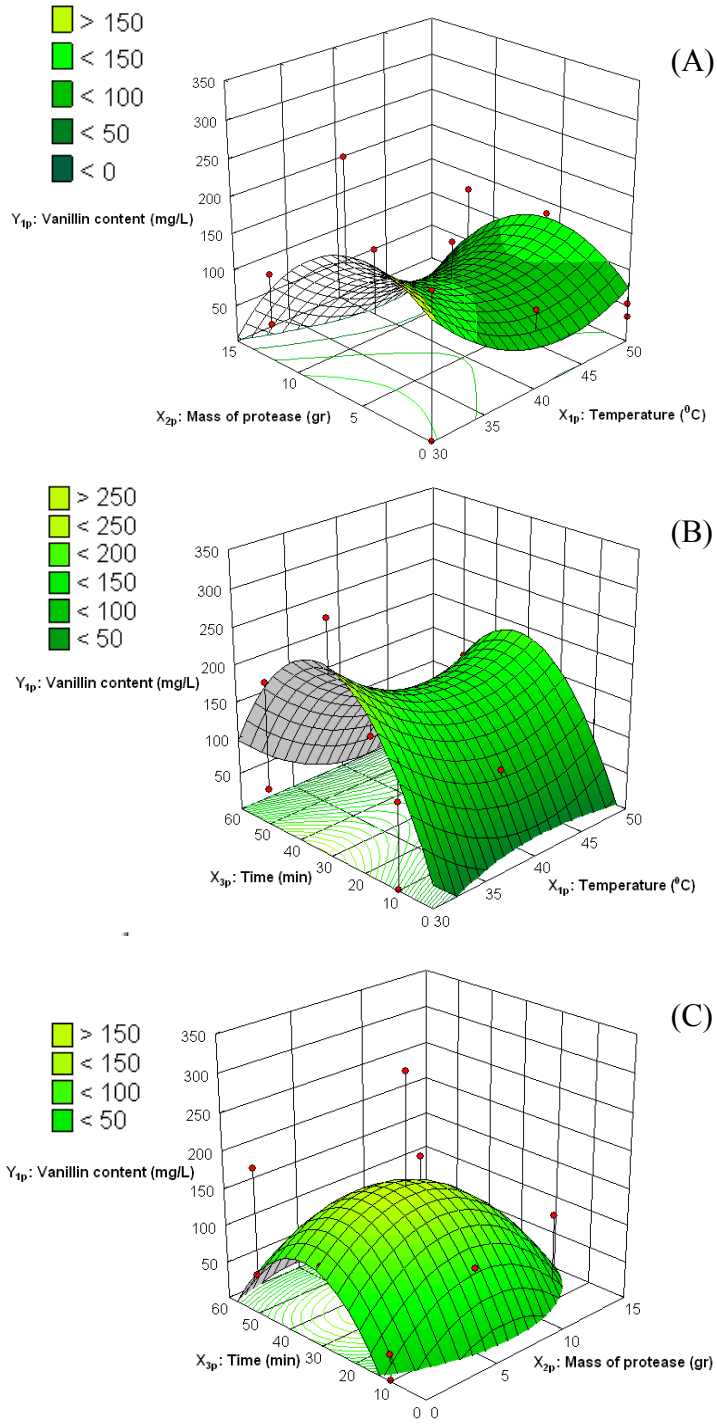

Figure 2. 3D-estimated response surface by plotting vanillin content (Y_{1p}) versus: (A), temperature (X_{1p}) and mass of protease (X_{2p}), extraction time was kept constant at 50 min; (B), temperature (X_{1p}) and extraction time (X_{3p}), mass of protease was kept constant at 6 gr (protease:substrate = 1:100); (C), mass of protease (X_{2p}) and extraction time (X_{3p}), temperature was kept constant at 40 °C.

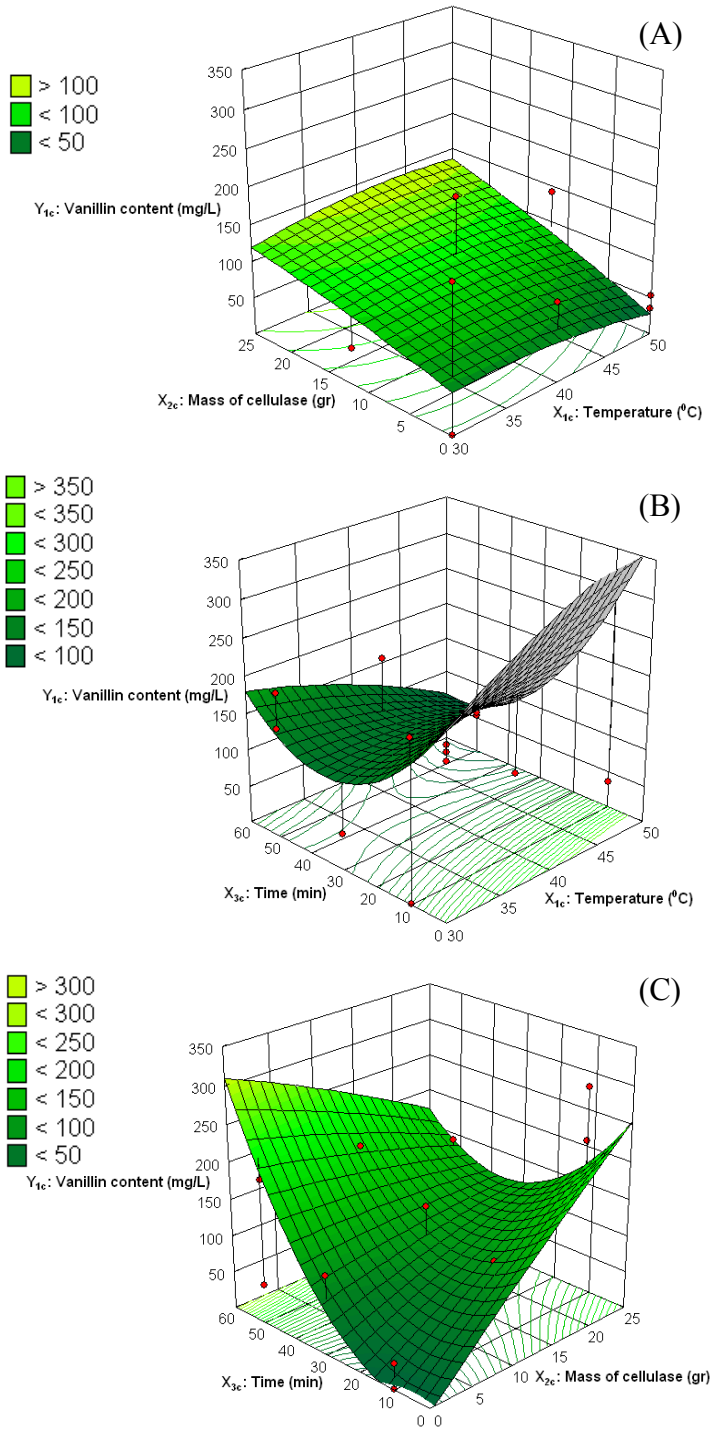

Figure 3. 3D-estimated response surface by plotting vanillin content (Y_{1c}) versus: (A), temperature (X_{1c}) and mass of cellulase (X_{2c}), extraction time was kept constant at 30 min; (B), temperature (X_{1c}) and extraction time (X_{3c}), mass of cellulase was kept constant at 24 gr (cellulase:substrate = 1:25); (C), mass of cellulase (X_{2c}) and extraction time (X_{3c}), temperature was kept constant at 30 °C.

The effects of the temperature and mass of cellulase on the vanillin content at 30 minutes extraction time is displayed in Figure 3A. An increase in the temperature and mass of protease reduced the vanillin content. The higher vanillin content was observed at the higher temperature of 45 °C and 25 gr of cellulase addition. The response was observed for the extraction temperature at any level of time at 24 grams of protease addition shown in Figure 3B. An increase of temperature increased the value of vanillin content while increasing the extraction time decreased the vanillin content. The higher vanillin content was observed at higher temperature of 50 °C on the initial stage of extraction time. The interaction effect of the cellulase addition and extraction time on the vanillin content at 30 °C in Figure 3C indicates an increase in the cellulase with shorter time for extraction.

3.4. *Optimum extraction parameters*

The predicted values of optimum vanillin content on critical values of extraction condition parameters and their influenced are shown in Table 2. Critical parameters to the obtained value of 118.15 mg/L by the addition of cellulase were 35 ºC of temperature with 22.89 of mass of cellulase (or 1:26.2 of enzyme-substrate ratio) during 38.52 minutes. Meanwhile, the critical parameters of protease addition treatment were found on 43 ºC with 6.02 gr of protease (or 1:99.67 of enzyme-substrate ratio) during 32.70 minutes for extraction time. On these critical parameters, the vanillin content can obtain 154.95 gr/L by protease addition. Gu *et al.* found that the optimal response of vanillin extraction yield was 7.62 mg/g [9]. The optimal conditions for vanillin extraction were 6 h of electrical heating time, 60 °C temperature and 33.5 mL of cellulase quantity.

Table 2. Predicted values of optimum vanillin content on critical values of extraction condition parameters and influenced parameters.

Parameters	Critical values	Optimum values of vanillin content regarding to the types of enzyme (mg/L)	Influenced parameter
Type of enzyme (-)	Protease	154.95	Extraction time
Temperature (°C)	43.00		
Mass of enzyme (gr)	6.02		
Time (min)	32.70		
Type of enzyme (-)	Cellulase	118.15	Mass of enzyme regarding to the extraction time
Temperature (°C)	35.00		
Mass of enzyme (gr)	22.89		
Time (min)	38.52		

4. Conclusions

The predicted optimum value of vanillin concentration was found at 154.95 mg/L by adding the protease on the extraction process, however, the coefficient of determination (R^2) was obtained at 0.6801. Although, the cellulase addition during extraction process was resulted lower optimum value of vanillin concentration (118.15 mg/L), the R^2 was provided higher value (0.8064) than thus the protease. These results indicated that the data obtained by the addition of cellulase provided a better accuracy of model than the addition of protease. Critical parameters to the obtained value of 118.15 mg/L by the cellulase addition were 35 ºC of temperature with 22.89 of mass of cellulase (or 1:26.2 of enzyme-substrate ratio) during 38.52 minutes.

References

[1] Gallage NJ and Møller BL 2015 *Mol. Plant* **8** 40
[2] Paramita V and Yulianto ME 2013 *J. Food Res.* **2** 65
[3] Sinha AK, Sharma UK and Sharma N 2008 *Int. J. Food Sci. Nutr.* **59** 299
[4] Rao SR and Ravishankar GA 2000 *J. Sci. Food Agr.* **80** 289

[5] Walton NJ, Mayer MJ and Narbad A 2003 *Phytochemistry* **63** 505
[6] Naidu MM, Kumar PVS, Shyamala BN, Sulochanamma G, Prakash M and Thakur MS 2009 *Food Bioprocess Technol.* **5** 527
[7] Odoux E, Chauwin A and Brillouet J-M 2003 *J Agric Food Chem* **51** 3168
[8] Perera CO and Owen E 2007 *Food Bioprocess Technol.* **3** 49
[9] Gu F, Xu F, Tan L, Wu H, Chu Z and Wang Q 2012 *Molecules* **17** 8753
[10] Ruiz-Terán F, Perez-Amador I and López-Munguia A 2001 *J. Agric. Food Chem.* **49** 5207
[11] Sampathu SRD, Naidu MM, Raghavan B, Thakur M, Kumar SPV and Prakash V 2009 Patent WO 2009031160 A1.
[12] Waliszewski KN, Ovando SL and Pardio VT 2007 *J. Food Eng.* **78** 1267
[13] Perussello CA, Zhang Z, Marzocchella A and Tiwari BK 2016 *Compr. Rev. Food Sci. Food Saf.* **16** 776
[14] Virot M, Tomao V, Ginies C, Visinoni F and Chemat F 2008 *J. Chromatogr. A* **1196 – 1197** 57
[15] Paramita V, Yulianto ME, Yohana E, Hanifah and Amjad MT September 29–30, 2015 *Int. Conf. of Chemical and Material Engineering* vol 1699 (Semarang: AIP Conf. Proc.) p 060009-1
[16] Lee SS, Kim C-H, Ha JK, Moon YH, Choi NJ and Cheng K-J 2002 *Asian Australas. J. Anim. Sci.* **15** 1725
[17] Barichievich EM and Calza RE 1990 *Appl. Environ. Microbiol.* **56** 43
[18] Lowe SE, Theodorou MK and Trinci APJ 1987 *Appl. Environ. Microbiol.* **53** 1216
[19] Ara KM and Raofie F 2016 *J. Food Sci. Technol.* **53** 3113
[20] Joshi VK, Chauhan A, Devi S and Kumar V 2014 *J. Food Sci. Technol.* **52** 4935
[21] Purwanto MGM 2016 *Procedia Chem.* **18** 127
[22] Milala MA, Shugaba A, Zanna H and Appollos B 2014 *ARPN-JST* **4** 433
[23] Breuil C and Saddler JN 1985 *Enzyme Microb. Technol.* **7** 327
[24] Montgomery DC 2017 *Design and Analysis of Experiments 9th*, (New York: John Wiley & Sons) p 230
[25] Box GEP, Hunter JS and Hunter WG 2005 *Statistics for Experimenters: Design, Innovation, and Discovery 2nd*, ed Barnett V, Hunter JS and Kendall DG (New York: John Wiley & Sons,) p 173
[26] Yılmaz FM, Karaaslan M and Vardin H 2015 *J. Food Sci. Technol.* **52** 2851
[27] Muyan C, Xiumei Z, Tianxiang G and Chao C 2006 *Chin J. Ocean Limnol.* **24** 300
[28] Pant G, Prakash A, Pavani JVP, Bera S, Deviram GVNS, Kumar A, Panchpuri M and Prasuna RG 2015 *J. Taibah Univ. Sci.* **9** 50
[29] Hoshino T, Ishizaki K, Sakamoto T, Kumeta H, Yumoto I, Matsuyama H and Ohgiya S 1997 *Lett. Appl. Microbiol.* **25** 70
[30] Pardo AG and Forchiassin F 1999 *Rev. Argent. Microbiol.* **31** 31
[31] Box GEP and Draper NR 1987 *Empirical model-building and response surface* (New York: John Wiley & Sons,) p 502
[32] Lima RB, Santos TBd, Vieira LGE, Ferrarese MdLL, Ferrarese-Filho O, Donatti L, Boeger MRT and Petkowicz CLdO 2013 *Carbohydr. Polym.* **93** 135

Acknowledgments
This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (Project Number: 138-27/UN7.5.1/PG/2015).