Mass Transfer Study on the Microwave Heating of Cured Vanilla Pods Extraction by applying Isolated Rumen Liquid of Cellulase

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***Abstract*—This research focused the study on the enzymatically extraction mass transfer of vanillin from cured vanilla pods. The novelty and the main innovations of this research is the development of hydrolysis-extraction process by using enzymatic extractor conducted with microwave. The enzyme of cellulose was isolated from rumen liquid. It can disrupt the shell wall of the pods in order to shift the equilibrium phase, therefore spurring the extraction rate and yield. The analysis of vanillin content was performed by using high performance liquid chromatography, following by the application of Matlab software in order to analyze the mass transfer of vanillin into continuous phase. The mass transfer coefficient of enzymatically extraction of cured vanillin content at 40 °C was found at 3.8116 s^{-1} which greater than the extraction without enzyme, found at 3.1523 s^{-1}.**

Keywords—cellulase; cell disruption; microwave; vanilla pods; vanillin

I. INTRODUCTION

Natural flavor of vanilla provided highly expensive price due to the limited production of vanilla pods all over the world with complexed process on-farm and off-farm stages [1]. Fresh green vanilla pods provided any aromatic flavor, however the favorable aroma develops by the curing process [2], [3]. This curing process required enzymes in order to disrup the shell wall of pods cellular compounds and serve the hydrolysis reaction of the vanillin precursor of glucovanillin by interacting with β-glucosidase [4].

The hydrolyzed glucovanillin performed many compounds with vanillin as one of the major of vanilla aromatic compounds [5]. Extraction of cured vanillin interested many researchers. In order to increase the extraction rate and yield, many studied proposed the application of enzymes addition. Many investigators studied the use of various enzymes such as pectinase and β-glucosidase on green vanilla pods [2] and cellulase and tea leaf enzymes on dried vanilla pods [1], [6]. Paramita and Yulianto promoted the application of isolated enzymes of rumen liquid since it contains the hydrolytic enzymes, such as cellulase, protease, pectinase [7], [8]. Moreoover, Trinci et al. stated that one of the most active polysaccharide-degrading enzymes was including cellulase which produced by ruminal anaerobic fungi [8], [9].

However, most of them applied the conventional heating with high risk of heat loss. Integrating the microwave on the extractor possibly decreased the heat loss [10]. Waliszweski et al. reported the kinetics of vanillin extraction by enzymes [6]. Moreover, Sampathu et al. patented the application of tea leaf enzymes for enhancing the vanilla flavor compounds [11]. However, the study of mass transfer on the vanillin extraction by the addition and without enzymes provided minimum reportase. Therefore, this work focused the study on the mass transfer of enzymatically extraction by applying cellulase (isolated from rumed liquid) conducted with microwave of vanillin content from cured vanilla pods.

II. MATERIALS AND METHODS

A. Materials

Cured green vanilla pods were collected from the region of Temanggung, Indonesia. Isolated rumen liquid enzymes of cellulase was obtained from the region of Semarang, Indonesia. Water and methanol of high performace liquid chromatography (HPLC) grade were obtained from Merck (Darmstadt, Germany). Vanillin standard was obtained from Sigma-Aldrich (Darmstadt, Germany).

B. Cellulose Isolation from Rumen Fluid

Rumen fluid extracted 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 sulphate, 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. The precipitate compound was obtained as enzyme, then 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) [12].

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C. Microwave Integrated Enzymatic Extraction

Six hundred grams of cured vanilla pods were blended. Six thousand millilitres of distilled water were placed in microwave interated bioextractor and set on the operation condition of extraction (30, 40, 50 °C). After the condition reached, the cut vanilla pods were added onto bioextractor. Then, cellulose (isolated from rumen fluid) was added with ratio to the substrate of 25:1. 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.

D. Vanillin content measurement

Concentration of vanillin was analyzed by using High Performance Liquid Chromatography Alliance 2695 with Photodiode Array Detector 2996 (Water Corporation, USA). One millilitre of samples was centrifuged for 10 min at 13,000 rpm and separated the filtrat. Ten microlitres of sample was then injected into HPLC. The column applied was Symmetry C18, 5 µm, 150×4.6 mm with 1 ml/min of flow rate. The mobile phases were H_2O and methanol with ratio of 60:40 % of water/methanol for ten min. Standard curve was obtained using vanillin.

III. Results and Discussion

A. Effect of Enzymatic Extraction Time and Temperature on the Vanillin Content and Glucose Content

Figure 1 presents the vanillin content with and without cellulase addition (0.04 of enzyme-substrate ratio) at pH 4 temperature of 30, 40 and 50 °C. During 50 min of extraction and without additional of cellulase at 40 °C can obtained 206,10 mg/L of vanillin content, however by cellulase addition, the vanillin content obtained 70,31 gr/L during the same extraction temperature. Decreasing the temperature into 30 °C resulted higher vanillin content (147,91 gr/L) and increasing the temperature into 50 °C resulted lower vanillin content (56,44 gr/L).

During 30 min of extraction time resulted lower vanillin content which was extracted with enzyme at 40 C (96,37 gr/L) than without enzyme addition (106,38 gr/L) at the same temperature. While adding the cellulase, increasing the extraction temperature into 50 °C, possibly increased the vanillin content until 222,62 gr/L. This result indicated that the application of enzyme effectively affect result of vanillin. Enzyme addition at 50° C with 30 min extraction time showed better result than applying longer extraction process.

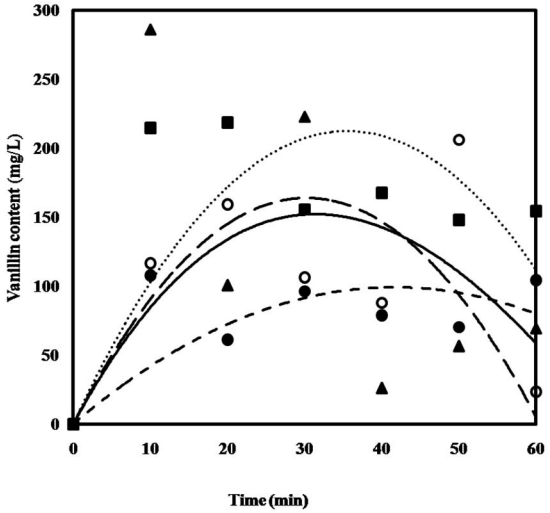

Fig. 1. Vanillin content on the extracted cured vanilla pods as a time function at pH 4: O (solid line), without enzyme, extracted at 40 °C; 0.04 of cellulase-substrate ratio: ■ (dot line), extracted at 30 °C; ● (short dash line), extracted at 40 °C; ▲ (long dash line), extracted at 50 °C.

B. Mass Transfer Rate on the Vanillin Liberation

The vanillin content extraction is regarded as a series of mass transfer phenomenon which is including the vanillin diffusion from inside of solid material into surface of solid material, the mass transfer of vanillin from surface of solid material into liquid solvent on the solid porous and the diffusion of vanillin into liquid solvent. Solid-liquid rate extraction depends on the vanillin diffusion from inside of solid material into surface of solid material and the mass transfer of flavonoid from surface of solid material into liquid solvent. If the differences of diffusion rate and mass transfer were almost the same, the extraction rate is determined by both of two processeses. However, if the differences of both rate can be considerable, then the extraction rate is determined by the slowest process rate [12].

Mass transfer rate of the vanillin diffusion from inside of solid material into surface of solid material is expressed in (1) [12], [13]:

$$N_{AS} = -D_e \cdot A \cdot \frac{dC}{dr} \quad (1)$$

where, N_{AS} = flux of mass (kg/s), D_e = diffusivity of solute (m^2/s), A = surface area of contact diffusion (m^2), C = solute concentration on the solvent (kg/m^3), r = distance of mass transfer (m). Following equation is the mass transfer rate of the vanillin from solid surface into the liquid solvent:

$$N_{AS} = K_{La} V (C_s - C) = \frac{dM}{dt} \quad (2)$$

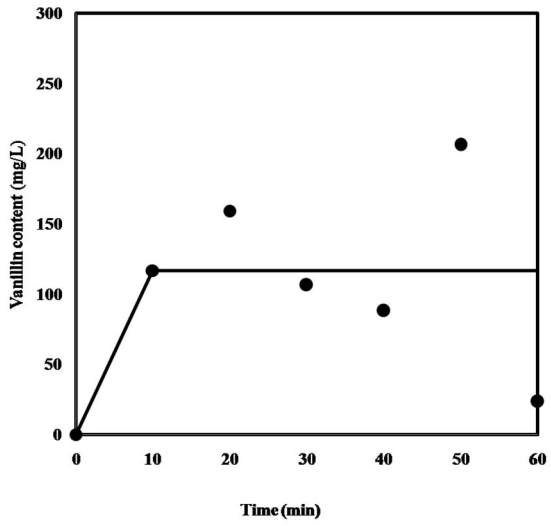

Fig. 2. Vanillin content extracted at 40 °C without enzyme addition as time function. ●, experimental yield and solid line, calculated yield.

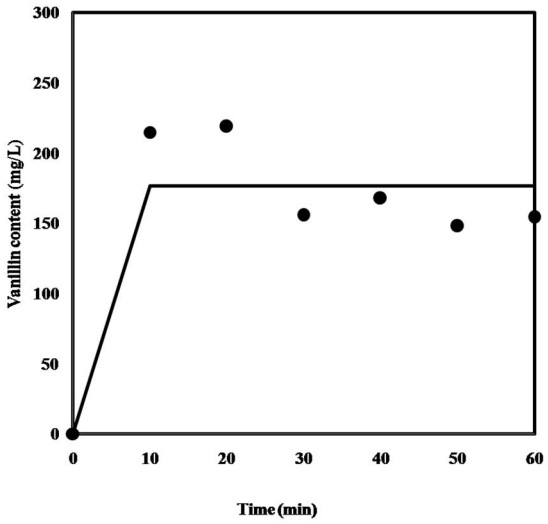

Fig. 3. Vanillin content extracted at 30 °C with celulase addition (enzyme-substrate ratio = 25:1) as time function. ●, experimental yield and solid line, calculated yield.

where, M = mass of transferred solute (kg), V = volume of solvent (m^3), t = time (s), Cs = solute concentration at the solid surface, which equilibrate with the solute concentration at the saturated solution (kg/m^3), K_{La} = volumetric mass transfer coefficient (s^{-1}).

Since the solid grain was prepared in very small size, the diffusion of vanillin from the solid surface perform rapidly and can be neglected. Therefore, mass transfer of flavonoid from solid into liquid is being decisive [14]. The second equation further diffferentiated in order to obtain the final equation of [12]:

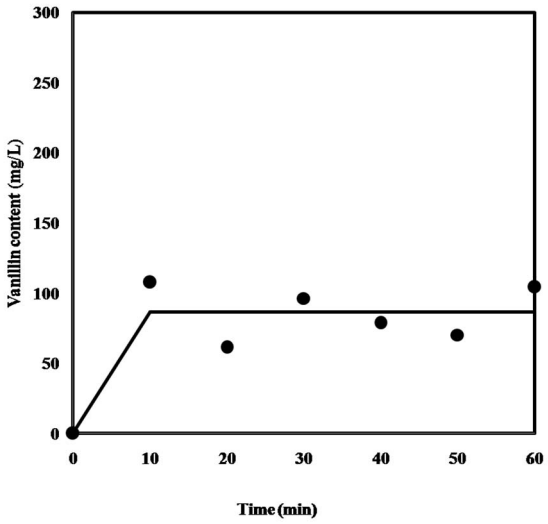

Fig. 4. Vanillin content extracted at 40 °C with celulase addition (enzyme-substrate ratio = 25:1) as time function. ●, experimental yield and solid line, calculated yield.

TABLE I. MASS TRANSFER COEFFICIENT AND SUM SQUARE OF ERRORS

Parameter		Mass transfer coefficient (s-1)	SSE (-)
Enzyme addition	*Extraction temperature (℃)*		
Without cellulase	40	3.1523	19,434.8796
Substrate : cellulase = 25:1	30	3.9491	5,064.1726
	40	3.8116	1,815.2678
	50	3.6332	53,655.2603

$$C = \frac{B}{A} - \frac{B}{A}e^{(-At)} \quad (3)$$

where, A = K_{La} and B = $K_{La}\left(\frac{m\cdot(C^0-C^\infty)}{V}\right)$. The relationship between the solute concentration in a saturated solution with initial solute concentration in the solid is C^0, the solute concentration in the rest of the sample at infinite time is C^∞ and initial mass sample is m.

Then, MATLAB (Mathworks Inc., United States) was applied to obtain the value of K_{La} by optimizing one variable. This optimization is performed to obtain the sum square of errors (SSE) or the smallest sum of the squared of the difference between the C count (C_{count}) with C trial (C_{trial}).

$$SSE = \sum(C_{count} - C_{trial})^2 \quad (4)$$

Model of mass transfer in enzymatic extraction of vanillin were verified by experimental data, as shown in Fig. 2 – Fig. 5. Whereas, Table 1 shows the value of mass transfer coefficient (K_{La}) and the sum square of errors (SSE) of experimental dan counted data. The lowest value of the sum square of errors indicated the best fit model to the experimental data. The

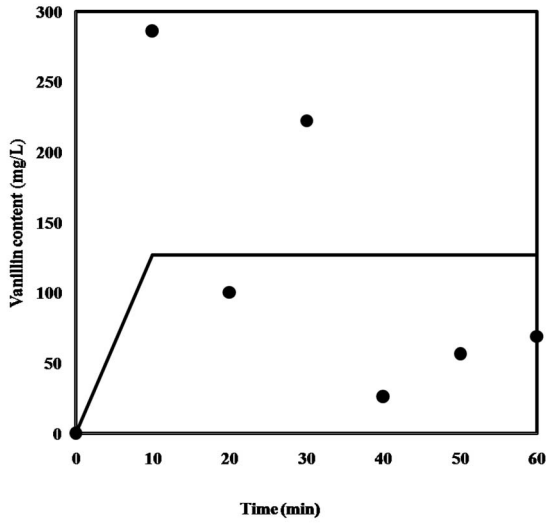

Fig. 5. Vanillin content extracted at 50 °C with celulase addition (enzyme-substrate ratio = 25:1) as time function. ●, experimental yield and solid line, calculated yield.

higher the mass transfer coefficient reflected the faster difussion rate of vanillin from solid surface into the liquid solvent.

Without addition of cellulase resulted the lowest value of mass transfer coefficient (3.1523 s^{-1}). Mass transfer coefficient increased (3.8116 s^{-1}) by the addition of 0.04 of cellulase-substrate ratio at the same temperature extraction condition (40 °C). Increasing the temperature into 50 °C decreased the mass transfer coefficient (3.6332 s^{-1}), while conversely. However, the lowest value of SSE at 40 °C with the enzyme addition (1,815 s^{-1}) indicated the most fit modelling to the experimental data (Fig. 4). The effective extraction time reached during 20 minutes extraction process, since longer extraction time obtained the decreasing of vanillin content, rather than increase. Paramita & Yulianto (2013) showed the longer extraction time, upto 12 h, in order to extract the vanillin content from the green vanilla pods by using electric heating. This condition showed the superior heating system of microwave. Moreover, Samun reported that the extraction by using electric heating obtained the lower value of KLa (0.0055 s^{-1}) than applying microwave heating [15].

IV. Conclusion

The mass transfer coefficient (KLa) of enzymatically extraction of cured vanillin content at 40 °C was found at 3.8116 s^{-1} which greater than extraction without enzyme (3.1523 s-1). Decreasing the temperature into 30 °C decreased the value of KLa (3.9491 s^{-1}). Short extraction time achieved (20 min) while applying microwave heating to the extraction process.

Acknowledgment

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References

[1] M.M. Naidu et al., "Enzyme-assisted process for production of superior quality vanilla extracts from green vanilla pods using tea leaf enzymes," Food Bioprocess Technol., vol. 5, pp. 527-532, November 2009.

[2] C.O. Perera and E. Owen, "Effect of tissue disruption by different methods followed by incubation with hydrolyzing enzymes on the production of vanillin from Tongan vanilla beans," Food BioprocessTechnol., vol. 3, pp. 49-54, December 2007.

[3] A.K. Sinha, U.K. Sharma, and N. Sharma, "A comprehensive review on vanilla flavor: extraction, isolation and quantification of vanillin and others constituents," Int. J. Food Sci. Nutr. , vol. 59, pp. 299-326, June 2008.

[4] E. Odoux, J. Escoute, J.-L. Verdeil, and J.-M. Brillouet, "Localization of β-D-glucosidase activity and glucovanillin in vanilla bean (*Vanilla planifolia Andrews*)," Ann. Bot-London, vol. 92, pp. 437-444, July 2003.

[5] T. General, V. Mamatha, V. Divya, and K.A.A. Appaiah, "Diversity of yeast with β-glucosidase activity in vanilla (*Vanilla planifolia*) plan," Curr. Sci., vol. 96, pp. 1501-1505, June 2009.

[6] K.N. Waliszewski, S.L. Ovando, and V.T. Pardio, "Effect of hydration and enzymatic pretreatment of vanilla beans on the kinetics of vanillin extraction," J. Food Eng., vol. 78, pp. 1267-1273, February 2007.

[7] V. Paramita and M.E. Yulianto, "Effect of β-glucosidase activity on the vanillin enzymatic formation by using rumen liquid for cell walls degradation," J. Food Res., vol. 2, pp. 65-69, March 2013.

[8] S.S. Lee, C.-H. Kim, J.K. Ha, Y.H. Moon, N.J. Choi, and K.-J. Cheng, "Distribution and activities of hydrolytic enzymes in the rumen compartments of hereford bulls fed alfalfa based diet," Asian Australas. J. Anim. Sci., vol. 15, pp. 1725-1731, January 2002.

[9] A.P.J. Trinci, D.R. Davies, K. Gull, M.L. Lawrence, B.B. Nielsen, A. Rickers and M.K. Theodorou, "Anaerobic fungi in herbivorous animals," Mycol. Res., vol. 98, pp, 129-152, February1994.

[10] M. Virot, V. Tomao, C. Ginies, and F. Chemat, "Microwave-integrated extraction of total fats and oils," J. Chromatogr. A., vol. 1196, pp. 57-64, July 2008 [ExTech 2008 – 10th International Symposium on Advances in Extraction Techniques].

[11] S.R.D. Sampathu et al., "An improved proces for the preparation of natural vanilla extract," WO2009031160 (A1), March 2009.

[12] Paramita V. et al., "Effect of immobile isolated enzymes from rumen liquid by using alginate matrices on the bay leaf extraction," AIP Conf. Proc., vol. 1699, pp. 060009-1–060009-8, September 2015 [2nd International Conference of Chemical and Material Engineering (ICCME) 2015].

[13] A. Ibarz and G. Canovas, "Unit operation in food enginering," Boca Raton: CRC Press, 2003, pp. 737.

[14] J.M. Smith, "Chemical engineering kinetics," Singapura: Mc. Graw Hill BookCo., Inc., 1981, pp. 65.

[15] Samun, "Volumetric mass transfer coefficient of natural dyes extraction from turmeric rhizome in stirred tank (*Koefisien transfer massa volumetris ekstraksi zat warna alami dari rimpang kunyit di dalam tangki berpengaduk*)," Ekuilibrium, vol. 7, pp. 17-21, 2008.

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