



# Economic Factor on the In Situ Vanillin Enzymatic Formation from the Green Pods Vanilla

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**Abstract** - This study proposed a feasibility study of an enzymatic hydrolysis-extraction of vanillin content and formation by using rumen fluid. The enzyme provided the capability for tissue disruption of vanilla green pods to avoid the curing process. The focus of this study was to evaluate the effect of hydration time of vanilla beans in water and enzymatic against the temperature. Green vanilla pods were applied for the direct enzymatic extraction of vanillin, while liquid rumen provide the cell wall degrading enzyme in order to support the destruction of cell wall by hydrolysis process. Short time hydrolysis of glucovanillin into vanillin possible occurs by the addition of liquid rumen. The enzyme from the liquid rumen can support the cell wall degradation and obtain the higher vanillin content (306 ppm) of mature vanilla pods at 30 °C. Moreover, the project of vanilla plantation is feasible to be applied with positive prospect. Primary economic analysis shows that vanilla plantation is a feasible project to be applied and developed.

**Keywords** –glucovanillin; vanillin; green vanilla pods; enzymatic extraction

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## 1. Introduction

The delicate and moderate sweet of vanilla, which is naturally extracted from cured tropical vanilla beans (*Vanilla planifolia* Andrews), allows the premium taste and flavour in the food and fragrance application. Walton et al. (2003) expressed that less than 1% of natural extracted vanilla of vanillin was produced annually. The major vanillin in the world market was sold less than US\$15 per kilo which prepared by chemical or biological process. While the natural one accepted at the fluctuate value in the range of US\$1200 and US\$4000 per kilo [1-4]. This expensive price of the natural extracted vanillin due to its complex aroma occur during both the on and post harvesting vanilla process.

In the mature green vanilla pods, the beans provided the precursor of vanillin, namely glucovanillin, with have no specific aroma of vanilla. The vanillin (4-hydroxy-3-methoxybenzaldehyde) can be occurring by the hydrolysis of glucovanillin by  $\beta$ -D-glucosidase activity during curing

process and resulting glucose and vanillin [5-8]. Curing process is contacting the aroma precursor and the  $\beta$ -D-glucosidase which probably located in separated tissue [6]. Therefore, the cell wall degradation is requiring to ensuring the contact [6,9].

The conventional curing process involves 6 months curing process to ensure the cell wall degradation and the hydrolysis of glucovanillin. Developing the conventional curing process is the crucial process in order to decrease the time and economic consumptions. Some researcher modified the curing process by implementing a commercial enzyme [10,11]. However, commercial enzyme provided an expensive cost of material, the novelty of this work was modifying the commercial enzyme by using rumen liquid. Selinger *et al.* presented that the rumen liquid obtained provided the advanced polysaccharide-degrading and hydrolytic enzymes, including, cellulases, xylanases, amylase, glucanases, amylases, pectinases and proteases [12]. The anaerobic rumen liquid enzyme activity promotes

3-6 times higher than the enzyme from aerobic fungi, *i.e.* *Aspergillus oryzae* [13]. It gave a potential development of rumen liquid enzyme for biotechnology industry.

This study proposed a feasibility study of an enzymatic hydrolysis-extraction of vanillin content and formation by using rumen fluid. The enzyme provided the capability for tissue disruption of vanilla green pods to avoid the curing process. The focus of this study was to evaluate the effect of hydration time of vanilla beans in water and enzymatic against the temperature.

## 2. Materials and Methods

### 2.1 Materials

Fresh vanilla beans were obtained from the subregion of Salatiga, Indonesia. The beans were kept at 4 °C until process. Rumen fluid, contains anaerobic enzyme of cellulase, protease and pectinase, were gained from the subregion of Semarang, Indonesia. Acetic acid, ethanol, and methanol of high performance liquid chromatography grade were purchased from Merck (Darmstadt, Germany).

### 2.2 Enzymatic Extraction

Cut vanilla beans (50 gr) of ~0.5 cm, 150 mL of distilled water and buffer phosphate (for controlling pH at 7) were placed in jacketed beakers and performed the extraction. The rumen fluid was added at 30 and 40 °C, the extraction were kept at 6, 12, 18, 24, 30 and 36 h. The reactions were kept in suspension by agitation using a magnetic stirrer. The analyses were carried out after the addition of ethanol to reach 47.5% v/v concentration in the enzyme reaction mixture, allowing 1,5 h to complete the extraction. Samples during extraction time were taken every 30 min. The content of vanillin and glucovanillin were quantified by using HPLC. In addition, the amounts of glucose were quantified by spectrofotometry analysis [14].

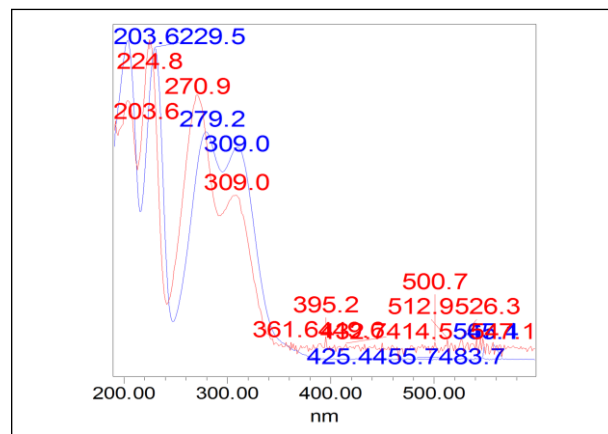
### 2.3 Economic Evaluation

The fixed capital investment, both the direct and indirect costs, was estimated from actual condition in Indonesia and calculated manually on Excel worksheets. The annual fixed capital investment was calculated by corresponding to 10-year life of the plant, 16 % of interest rate, and 10 % of depreciation. The reference year was 2012 and 8 working hours per day were assumed. The working capital investment was calculated according to the financial cash flow analysis [15,16]. Its annual working capital represented by multiplying the gross with the interest rate and added value tax (15 %). This economical analysis is the preliminary design for the future techno-economic analysis on the direct vanillin formation and extraction process.

## 3. RESULTS AND DISCUSSION

### 3.1 Determination of Glucovanillin and Vanillin by using High Performance Liquid Chromatography

The determination of glucovanillin and vanillin were presented on the Figure 1. Glucovanillin can be detected on 12.46 min and vanillin was detected on 19.30 min. This result affected on the final result of glucovanillin, which

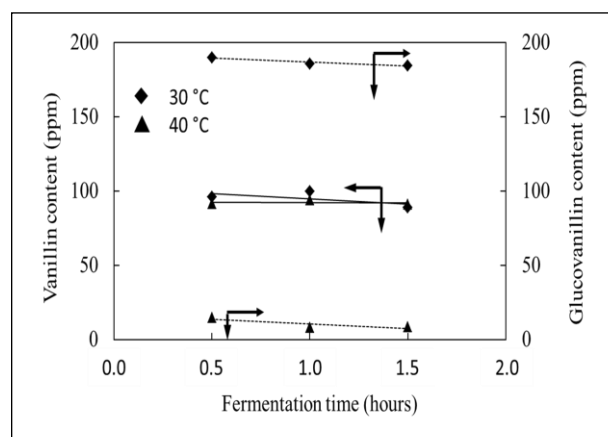


**Figure 1.** UV-Vis spectrum of glucovanillin, peak of time retention at 12.46 min (red line), and vanillin, peak of time retention at 19.30 min (blue line)

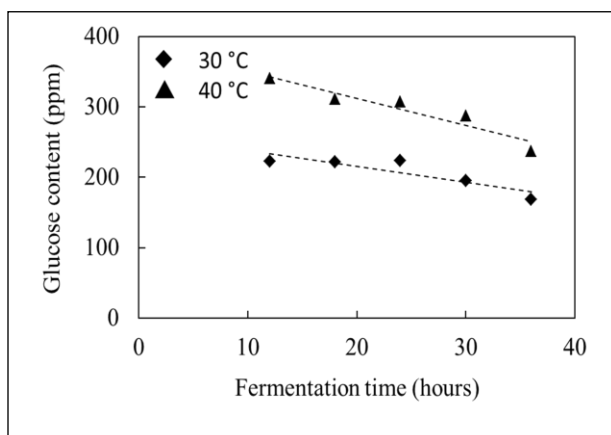
were the glucovanillin content nearly 0 ppm during fermentation of vanillin. On 12.9 min, there is a dominance peak appeared, it has possibility of the peak of threonic acid. Sinha *et al.* reported the application of many devices in order to analyze the vanillin content and high performance liquid chromatography (HPLC) provided the appropriate analysis of active compounds in the natural extracts due to its sensitivity and precision [17].

### 3.2 Effect of Extraction Time and Temperature on the Vanillin and Glucovanillin Content

Ruiz-Terán *et al.* analyzed the vanillin content treated by using Celluclast and extracted from vanilla pods by using water (1.17 g/100 g of dry pods) and ethanol (2.66 g/100 g of dry pods) [10]. This result shows that ethanol effect the extraction of vanillin. This study are in agreement about the effectiveness of ethanol for extracting the vanillin, regarding on their temperature. Figure 2 shows the vanillin and glucovanillin content regarding to its extraction time and temperature.



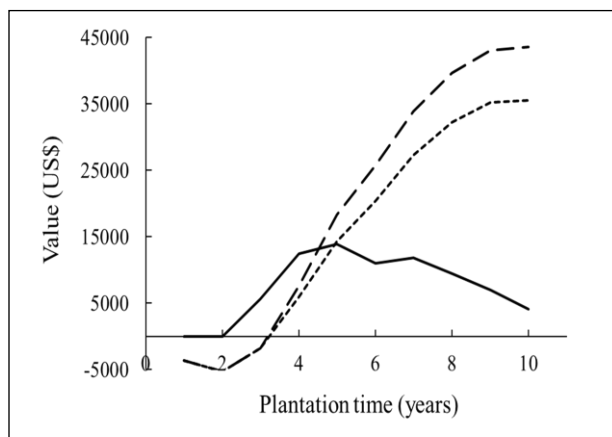
**Figure 2.** Effect of extraction time and temperature on the vanillin content and glucovanillin content at pH 7 and temperature of 30 °C (◆) and 40 °C (▲). Vanillin content: solid line, glucovanillin content: dash line



**Figure 3.** Effect of fermentation time and temperature on the glucose content at pH 7 and temperature of 30 °C (◆) and 40 °C (▲)

The initial vanillin content on the green pods was found 10.0 ppm. By treating the vanilla pods at 30 °C, the vanillin content was found in the range of 302–306 ppm during fermentation stage. However, it was decreased below 100 ppm during extraction period. While treating at 40 °C, it was found the increasing of vanillin content after 36 hours fermentation, 42.4 ppm, into 91.5 ppm for half hours of extraction period. This result shows an anomaly since there is no exact mechanisms for this condition. However, there are some possibility that can support them. It could be indicated that dissolved vanillin in ethanol vaporized easily than dissolved vanillin in water or it may form another substances by treating at 30 °C. When treating at 40 °C, the vanillin content was found lower during fermentation. It may show that the β-glucosidase has a lower activity at this temperature, however it could be extracted better by using ethanol. Yapi *et al.* reported that purified β-glucosidase stable at 37 °C and pH of 5,0–6,0 [18].

Moreover, Figure 3 also reported that there is some amount of glucovanillin extracted at 40 °C (< 15 ppm) while



**Figure 4.** Cash flow of vanilla plantation for 10 years. Cash in (gross revenue): solid line; gross profit: long dash line; net profit: short dash line

**Table 1.** Cost used in the evaluation

Chemicals nutrients	Value	Other costs	Value
Compost	0,10 US\$/kg	Electricity	82,6 US\$/MWh
CO(NH <sub>2</sub> ) <sub>2</sub>	0,16 US\$/kg	Depreciation	10% of fixed capital
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0,16 US\$/kg		
P <sub>2</sub> O <sub>5</sub>	0,29 US\$/kg		
Ca(H <sub>2</sub> PO <sub>4</sub> )	0,29 US\$/kg		
KCl	0,16 US\$/kg		
CaO	0,16 US\$/kg		
Pesticide/fungi side	7,78 US\$/L		

nearly 200 ppm of glucovanillin could be extracted at 30 °C. Overall of this result may indicate that 30 °C provide better temperature for hydrolysis-fermentation and extraction from vanilla green pods.

**3.3 Effect of Fermentation Time, Extraction Time and Temperature on the Glucose Content**

Figure 3 shows the effect of fermentation and extraction time, respectively, with regards to the temperature and the glucose content. By carrying out the green vanilla pods at 30 °C, the vanillin content during extraction time (168 ppm at 36 hours) shows higher than its content during fermentation period (1057 ppm at 0,5 hours). This condition indicated that ethanol have a significant effect on glucose extraction and it also shows better solubility of glucose in ethanol comparing its solubility in water.

**3.4 Economical Analysis**

Figure 4 shows the cash flow of vanilla plantation for 10 years life of the plant on 1 hectare of harvesting area. The solid line represents the cash in (gross revenue), while the long and short dash lines refer to the cumulative value of the gross and net profit, respectively. The difference between gross dan net profit indicated the production cost during plantation. The optimal production can be found at the 5-year life of the plant, as showed by the gross revenue.

Table 1 shows the economical analysis for 1 hectare of harvesting area, it was obtained that the Internal Rate of Return (IRR) at 37.19% with Return of Investment (ROI) on 4-year life of the plant. The project can be included as feasible to be executed when the value of IRR greater than 16 % of interest rate (Umar 2000). The value of Break Even Point (BEP), equal with the product weight of vanilla was found on 1,299 kg and Net Present Value (NPV) was found on US\$ 13,447. The benefit cost ratio (B/C Ratio) was obtained on 1.75. It indicated that this project meet the minimal requirement of B/C ratio at 1.00. All of these valuea were possible to be applied and developed for green pods vanilla price of US\$ 15.6.

**4. Conclusions**

Short time hydrolysis of glucovanillin into vanillin possible occurs by the addition of liquid rumen. The enzyme from the liquid rumen can support the cell wall degradation and

obtain the higher vanillin content (306 ppm) of mature vanilla pods at 30 °C. Moreover, the project of vanilla plantation is feasible to be applied with positive prospect. Primary economic analysis shows that vanilla plantation is a feasible project to be applied and developed.

## 5. Acknowledgement

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