The Effect of Bean Size and Curing Process on Aroma Profile and Vanillin/Glucovanillin Content of Indonesian Cured Vanilla Beans

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Abstract: A cured vanilla bean produced in Indonesia has lower quality than that produced in other countries, which may be associated with improper maturity of vanilla bean and curing process. The quality of vanilla is dependent on its aroma and vanillin content. The aim of this research was to evaluate the effects of fresh vanilla bean size (weight and length) on aroma profile and vanillin/ glucovanillin of cured vanilla bean. Curing condition was conducted under 90ºC killing temperatures and sun drying period (dried to 50% of initial beans weight). Aroma profile was evaluated using sensory quantitative descriptive analysis and vanillin content determined by high performance liquid chromatography. The results showed that different size of vanila beans with same condition of curing process gave different aroma profile. Vanilla beans with size less than 8 grams or 12.3 cm length of vanilla bean did not have good aroma vanilla characteristics. The highest vanillin content was found in vanilla with size of bean more than 15 grams or 19.6 cm length of vanilla bean, and characterised as vanillin aroma.

1 INTRODUCTION

Indonesia is one of the biggest vanilla producers in the world beside Madagascar, Mexico, India and Africa. However, the price of Indonesian vanilla beans tends to be the lowest (US$12.592 per ton) over vanilla beans produced from other countries (FAO, 2009). fruit maturity (indicated by bean size) and curing condition seems to be a key stage affecting the aroma compounds of the vanilla bean; thus, improper harvesting time and curing process may contribute to production of undesired aroma on the Indonesian vanilla beans, such as dry smoky or woody aroma (Adedeji et al., 1993).

Mature green vanilla beans contain precursors of flavor and aroma compounds, and these compounds are released during curing process. The curing process induces the enzymatic reaction which converts glucovanillin to vanillin. In green vanilla beans, the concentration of glucovanillin was found around 14% dry weight basis (Havkin-Frenkel and Belanger, 2010). Many studies have investigated the effects of various curing conditions on the hydrolytic activity of glucovanillin (Dignum et al., 2002; Havkin-Frenkel et al., 2004; Pérez Silva et al., 2011). In a study, vanillin concentration was found to increase during curing process from 8418 ppm to 22361 ppm (Pérez Silva et al., 2011). Vanillin content in cured vanilla beans is an important analytical parameter, which indicates a strong relationship between curing condition and vanilla quality. Numerous studies have identified hundred volatile compounds present in vanilla bean, and their presence is responsible for the aroma profile of vanilla (Adedeji et al., 1993; Pérez-Silva et al., 2006; Sostaric et al., 2000). However, vanillin is the major volatile compound in vanilla bean (Sreedhar et al., 2007; Pardio et al., 2009; Zhang and Mueller, 2012). Many studies have been conducted to determine the optimal conditions to obtain a good quality of cured vanilla bean (Dignum et al., 2002; Van Dyk et al., 2010).

Under ripe or immature green vanilla bean will produce low quality of cured vanilla bean compare with mature vanilla bean. Van Dyk *et al.* (2010) reported that physiological age of vanilla bean during harvesting is important factor affecting vanilla quality. General indicators of fully mature vanilla beans are green-yellow color, cylindrical shape and length 10-25 cm and witdh of 1-1.5 cm (Rao dan

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Ravishankar 2000). Medina *et al.* (2009) mentioned the importance of sorting and grading on the harvested vanilla bean. The size and appearance become very important because it is related with the aroma produced from their Vanillin component during curing process.

Based on information as mentioned above, vanilla quality is heavily affected by maturity level based on size parameter of vanilla bean or pod and curing process. In this paper, we study on the effect of vanilla bean size (weight and length) on the quality of cured vanilla bean evaluated from aroma profile and vanillin / glucovanillin contents.

2 MATERIAL AND METHODS

2.1 Materials

Green vanilla beans were obtained from vanilla producers in Alor, Indonesia and commercial Madagascar vanilla were supplied by Ogawa Co., Japan. Chemicals for HPLC analysis were of analytical grade (Merck, Germany), while aroma references (vanilin, asam asetat, *guaiacol,* eugenol, *cinnamaldehyde*, *1-octen-3-ol*, *2,4-decadienal*,*C-8 acid*, furfural, etil asetat, etil propanoat, isoamil asetat, fenil etil alkohol, *alcohol C-10*) for sensory analysis were also provided by Ogawa Co. Japan.

2.2 Method

Fresh vanilla beans were graded into 4 classes based on weight: (A) less than 8 gram, (B) 8-11 gram, (C) 12-15 gram and (D) more than 15 gram. Length of vanilla beans were measure individually to determine range of length of vanilla bean in each class.

The beans were killed by immersion in hot water at 90ºC for 3 mins. After killing, all bean groups were wrapped with three layers: black cotton cloth, black flannel cloth and black plastic sheet (from inner to outer). For sweating stage, the wrapped beans were put in a separated box (using styrofoam box) and kept for 24 hr. Next, the vanilla beans were sun-dried for 5 hr per day until reaching 50% weight reduction. Prior to sun drying, the beans were wrapped with two layers, e.g., black cotton cloth and black flannel cloth and placed in separated styrofoam box for the next day. After this stage, all bean samples were subjected to subsequent slow drying. In this process, the beans were placed on trays in a ventilated room to continue the drying process until the bean weights reached 20-22% of their initial weight. After slow drying, the beans were bundled and then stored in a wooden box at ambient temperature for 3 months for conditioning process. It was noted that sweating, slow drying and conditioning process were conducted at the same condition for each group of vanilla beans.

2.2.1 Sensory Analysis

The method used for the sensory evaluation was quantitative descriptive analysis (QDA). The aroma quality of cured vanilla beans was evaluated by 13 trained panellists of Bogor Agricultural University (9 women and 4 men, aged from 21 to 28 years old), previously selected from 41 candidates. The panellist was trained for 1 month by rating test and focus group discussion for vanilla aroma. Approximately 1 g of samples (only seeds have taken) were served in dark vial (5 mL capacity), coded and presented randomly to panellists. They evaluated the aroma (sweet, sour, phenol-like, spicy, hay-like, liquid/whisky-like, and woody) in samples with reference odours (Table 1) and scored aroma descriptor on an intensity scale of 0 – 10 indicating "none" to "strong". All sensory evaluations were performed in individual sensory booths at room temperature (26-27ºC). The sensory evaluation test was conducted in triplicate.

Table 1: Description of aroma references.

References	Description
Vanilin	Sweet
Asam asetat	acid
Guaiacol, eugenol	Phenol like
Cinnamaldehyde	spice
1-octen-3-ol, *2,4-decadienal*, *C-8 acid*	Hay-like
Furfural, etil asetat, etil propanoat	Liquid or whisky-like
Fenil etil alkohol, *alcohol C-10*	woody

2.2.2 Determination Vanillin & Glucovanillin

Sample Preparation. Cured vanilla beans (approximately 200 mg) were suspended in 10 mL distilled water, added with 0.5 mL of 18 M sulphuric acid and thoroughly mixed. The mixture was then incubated in a waterbath at 60ºC for 3 h to hydrolyze the glycosides, cooled in room temperature and added with 2 mL of 9 M KOH to neutralize the mixture solution (pH adjusted to 6-7). Then, 10 mL of n-hexane and dicloromethane (1:1, v/v) was added to extract the vanillin and shaken. The upper organic phase was recovered, while the remaining aqueous layer was extracted three times more with 10 mL of

n-hexane and dichloromethane (1:1,v/v). The combined organic phase was dried over anhydrous Na_2SO_4 and filtered. The filtrate was dried using N_2 gas. The dried analyte was adjusted to 2 mL by mobile phase solvent and filtered using membrane filter 0.45 µm. The filtrate obtained was subjected to HPLC and the result was calculated as total vanillin of the samples. Free vanillin was analyzed using the above procedure but without acid hydrolysis and neutralization.

HPLC Analysis. Vanillin concentrations in the hydrolyzed extracts from cured vanilla beans were determined using HPLC Shimadzu LC-6A (Shimadzu, Japan). HPLC is operated at room temperature with the condition of column C18 (15 cm, 4.6 mm i.d., 5 µm, Zorbak Eclipse XBD-C18, Agilent, USA). The mobile phase consisted of 10% H_2O, 30% acetonitrile, 60% methanol. The separation was performed under isocratic condition at a constant flow rate of 0.5 mL/min. Detection was performed at 271 nm with a UV-Visible detector SPD-10AV (Shimadzu, Japan). The injection volume was 20 µL of sample. Vanillin standard solutions, 5-100 $\mu g.mL^{-1}$ were prepared to obtain a linear calibration curve.

2.2.3 Statistical Data Analysis

Analysis of variance (ANOVA) was performed to evaluate significant differences in aroma profile among vanilla samples ($p<0.05$) using MINITAB version 2016. Principal component Analysis was performed using XLSTAT version 2014.

3 RESULT AND DISCUSSION

3.1 Vanilla Bean Size

Maturity level indicated by weight of individual vanilla bean affected of aroma profile of cured vanilla bean. Vanilla bean size was categorised by 4 groups based on weight: A) less then 8 gram, (B) 8-11 gram, (C) 12-15 gram dan (D) more than 15 gram. The length of vanilla beans were ranging from 12.3 cm – 19.6 cm. Table 2 shows data of size (length and weight) of vanilla bean used in this research. The smallest size of vanilla bean was less than 8 gram equivalent with 12.3 cm length (Table 2), while the biggest size was more than 15 gram that equivalent to 19.6 cm length.

Table 2: Vanilla bean size.

Name Group	Range	Average of weight (g)	Average of length (cm)
A	< 8 gram	5.16	12.3 ± 1.8
B	8-11 gram	9.52	15.5 ± 1.3
C	12-15 gram	13.55	17.6 ± 1.3
D	>15 gram	19.09	19.6 ± 1.5
A	< 8 gram	5.16	12.3 ± 1.8

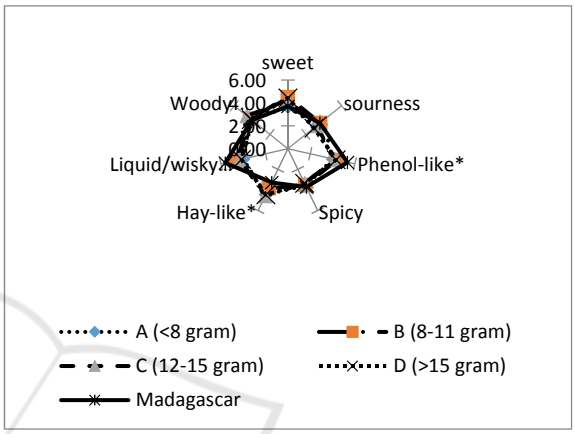

Figure 1: Aroma profile of 4 groups of cured vanilla bean. Note: *significant different ($p<0.05$).

The results in figure 1 shows that bean size (weight and length) significantly effect on aroma profile of cured vanilla beans. Significant different were found in phenol like, and hay like. Cured vanilla bean from fresh vanilla bean weight less than 8 gram had lower sweet aroma compare to the others. It means that fresh vanilla bean weighs less than 8 grams or length less than 12.3 cm has not fully ripe or optimum condition to produce sweet aroma because flavour precursor for vanilla was not developed yet. During this time, some of Indonesia's vanilla bean farmers harvested the vanilla bean in under-ripe or immature condition to avoid theft and to reduce the production cost. They harvested vanilla bean at once, mixed mature and immature vanilla bean. It resulted in low quality of Indonesian vanilla bean compared to vanilla from other countries such as the vanilla of Madagascar. Immature fresh Vanilla bean has incomplete flavor compound that responsible for targeted flavors, such as sweet and vanillin flavor, during the curing process (Ranadive 2006). The vanilla bean more than 15 gram had sweet aroma higher than the other size, although not significantly different. Mature vanilla bean produce aroma profile vanilla, sweet and creamy (Ranadive 2006).

3.2 Vanilin/Glukovanilin Contents

Vanilin is an important flavour compound quality parameter of cured vanilla bean as the result of hydrolysis of glucovanilin by β-glukosidase. In general, the vanillin concentration in cured vanilla bean on average is 1.4-3.0% (Gassenmeier *et al*. 2008; Cicchetti dan Chaintreau 2009). Cured Vanilla bean with a high concentration of Vanilin (2.0-3.0%) is a high-quality of vanilla. Concentration levels of vanillin in cured vanilla bean depend on several factors, one of which is the level of maturity of the fresh green vanilla bean during harvesting. This relates to the concentration of glucovanillin in the fresh vanilla bean as a precursor to the flavor compounds of vanillin. Tokoro et al. (1990) reported that the highest content of Glucovanillin is in mature vanilla fruit. In this research the vanillin compound was analyzed using HPLC UV-Vis and detected at the 3rd minute. The results showed that vanilla bean weight more than 15 grams had a higher concentration of vanillin compared to three others size of vanilla bean (Figure 2). The content of vanillin in this study was still relatively low in the range of 200-4000 ppm compared to the vanillin content of Mexican vanilla, Madagascar (Pérez-Silva *et al*. 2011; Pardio *et al*. 2009).

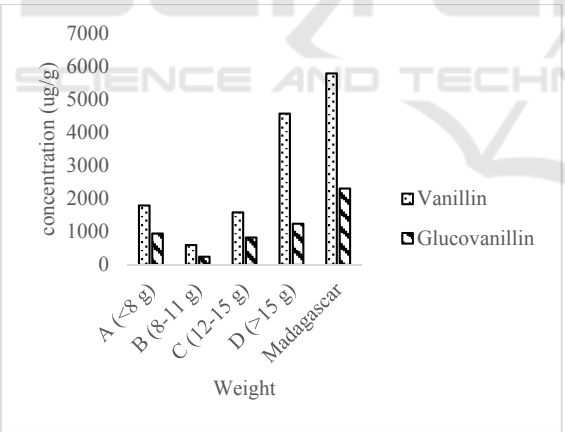

Figure 2.

The results showed that vanilla bean with size less than 15 grams on average had a lower vanillin content than the size of more than 15 grams and Madagascar vanilla. It is because Glucovanillin content has not formed maximum level due to low maturity level (immature). Glucovanillin is a dominant flavor precursor found in cured vanilla bean. Van Dyk et al. (2014) mentioned that Glucovanillin began to increase in the 20th week after pollination and achieved maximum concentration after 40 weeks after pollination. The physical condition as bean size became critical criteria in determining the optimum harvest time of vanilla bean for further processing of curing. Generally, Indonesian vanilla farmers do not pay attention to the size of vanilla fruit at the harvest time because usually, farmers harvest the vanilla bean at once without the selection of fruit one by one. It causes the low quality of vanilla beans from Indonesia compared to the other countries. Another factor that affected vanillin/glucovanillin content is the curing process, such as killing and drying methods.

3.3 Mapping of Cured Vanilla Beans Characteristics by Principal Component Analysis

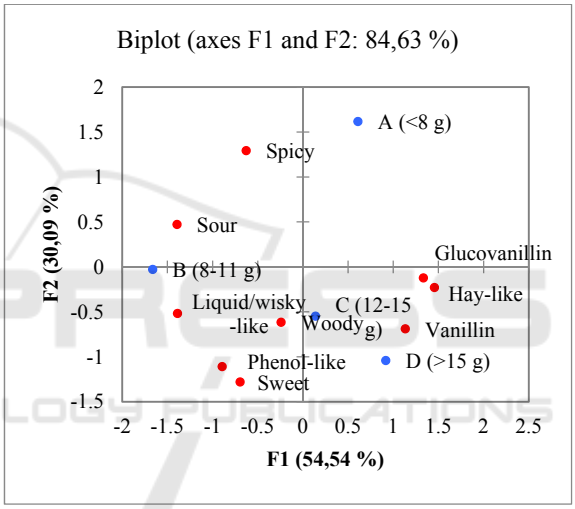

Figure 3: PCA of cured vanilla bean with different size.

Mapping and correlation between cured vanilla bean based on different size (weight) with aroma profile and vanillin/glucovanillin content was described using biplot graph from principle component analysis (PCA) as can be seen in Figure 3. Figure 3 shows mapping of characteristics cured vanilla bean from 4 different size of green vanilla bean as raw material. The result of PCA explained 84.63% of the total variance (F1=54.54% and F2=30.09%). The PCA shows that fresh vanilla bean with different size produce different characteristics of cured vanilla bean. Vanilla A (weight less than 8 grams) does not shows any dominant characteristics in sensory or aroma profile and vanillin/glucovanillin content. Vanilla B (weight 8-11 grams) has dominant characteristics of *liquid/whisky-like, phenol-like* dan *woody,* while cured vanilla C (12-15 grams) and D (more than 15 grams) were noted by

vanilin/glucovanilin content, and *hay-like* aroma. This result research clearly clarify that the immature vanilla bean indicated by small size less than 8 grams or length less than 12.3 cm would produce low quality of cured vanilla bean with less or no aroma identity. Many factors are involved in providing good quality of cured vanilla bean, including the level of fruit maturity, environmental condition, curing process, and others. In this study, the size of the vanilla bean was a good indicator of the maturity level of vanilla bean.

PCA also can be used to analyse correlation between tested parameters. PCA also can be used to analyse the correlation between tested parameters. A Positive correlation can be seen from adjacent criteria or parameters, while a negative correlation from the data or parameter position in the opposite side or diagonal quadrant. Based matrix correlation (data do not shown), there was positive correlation between glucovanillin with vanillin (r = 0.872), hay like aroma (r = 0.846). Negative correlations were found between vanillin with sour (r = -0.918); glucovanillin with sour (r = -0.986); glucovanillin with *liquid/whisky-like* (r = -0.869).

A positive correlation between glucovanillin with vanillin (r = 0.873) means that higher glucovanillin caused the higher vanillin concentration. High or low concentration of vanillin in cured vanilla bean depends on the concentration of Glukovanilin in fresh vanilla bean. Vanillin obtained from hydrolysis of glucovanillin by enzyme of β-glukosidase during curing process. According to Van Dyk et al. (2014), the concentration of Glucovanillin in vanilla bean highly depends on the level of maturity. Some result researches suggested that the maximum concentration of glucovanillin in fresh vanilla bean occurs at week 40 after pollination. This research result emphasizes that the level of maturity was highly significant in vanillin concentration produced during the curing process.

Glucovanillin also has a positive correlation with hay-like aroma (r = 0.846). Hay like is an undesirable aroma in the cured vanilla bean. High glucovanillin concentration in cured vanilla bean caused by less intense hydrolysis to convert glucovanillin into vanillin during the curing process. Hydrolysis of glucovanillin highly depends on enzyme β-glucosidase activity and water availability in the vanilla bean. The moisture content of cured vanilla bean was 13-14% wb which was relatively lower than the optimum moisture content of 25-30% (Brunschwig *et al.* 2012). It means intense hay-like aroma in cured vanilla bean found in this research was due to the low moisture content of vanilla bean. Takahashi et al. (2013) reported that cut split vanilla bean with the low moisture content of 11% had a higher hay-like aroma intensity than vanilla red whole beans with a moisture content of 25%.

4 CONCLUSION

Fresh vanilla bean size has a significant effect on the aroma profile and the vanillin content in the cured vanilla bean. Vanilla bean size less than 8 grams or length of less than 12.3 cm produces a low quality of cured vanilla bean in terms of aroma profile as well as the content of vanilin/glucovanilin. The size of the vanilla bean more than 17 cm or weigh more than 15 grams is recommended to the Indonesian vanilla farmer as the physical criteria of the fruit to harvest the vanilla bean to produces good characteristics of cured vanilla bean.

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REFERENCES

[FAO] Food and Agriculture Organization of the United Nations. 2009. Vanilla Post harvest Operation. 2009-06-16.

Adedeji J, Hartman TG, Ho C. 1993. Flavor characterization of different varieties of vanilla beans. *Perfum Flavor.* 18:115–133 Ranadive AS.2006. Inside look: Chemistry and biochemistry of vanilla flavor. *Perfum Flavor*. 31:38-44.

Brunschwig C, Rochard S, Pierrat A, Rouger A, Senger-Emonnot P, George G, Raharivelomanana P. 2016. Volatile composition and sensory properties of Vanilla ×tahitensis bring new insights for vanilla quality control. *J Sci Food Agric.* 96(3):848-858.

Brunschwig C, Senger-Emonnot P, Aubanel M-L, Pierrat A, George G, Rochard S, Raharivelomanana P. 2012. Odor-active compounds of Tahitian vanilla flavor. *Food Res Int.* 46:148-157.

Cicchetti E, Chaintreau A. 2009. Quantitation of the main constituents of vanilla by reverse phase HPLC and ultra-high-pressure-liquid-chromatography with UV detection: Method validation and performance comparison. *J Sep Sci*. 32(17):3043-52.

Dignum MJW, Kerler J, Verpoorte R. 2002. Vanilla curing under laboratory conditions. *Food Chem.* 79(2):165-71.

Gassenmeier K, Riesen B, Magyar B. 2008. Commercial quality and analytical parameters of cured vanilla beans

(Vanilla planifolia) from different origins from the 2006–2007 crop. *Flavour Fragr J*. 23:194-201.

Havkin-Frenkel D, Belanger F. 2011. Handbook of vanilla science and technology. Willey-Blackwell. hlm 142-148.

Medina J, Rodríguez G, García H, Rosado T, García M, Robles V. 2009. Vanilla: post-harvest operations. *Food Agric Organ United Nations Publ*. 2009:1-50.

Pardio VT, Mariezcurrena MD, Waliszewski KN, Sánchez V, Janczur MK. 2009. Effects of killing conditions of vanilla (*Vanilla planifolia*, Andrews) pods during the curing process on aroma composition of pod ethanol extract. *Int. J. Food Sci. Technol.* 44(12):2417-2423.

Pérez Silva A, Gunata Z, Lepoutre JP, Odoux E. 2011. New insight on the genesis and fate of odor-active compounds in vanilla beans (*Vanilla planifolia* G. Jackson) during traditional curing. *Food Res Int*. 44(9):2930-2937.

Rao SR, Ravishankar GA. 2000. Vanila flavour: production by conventional and biotechnological routes. *J Sci Food Agric*. 80(3):289-304.

Sreedhar RV, Roohie K, Venkatachalam L, Narayan MS, Bhagyalakshmi N. 2007. Specific pretreatments reduce curing period of vanilla (*Vanilla planifolia*) beans. *J. Agric. Food Chem*. 55:2947-2955.

Sostaric, T., Boyce, M.C., and Spickett, E.E. 2000. Analysis of the volatile components in vanilla extracts and flavorings by solid-phase microextraction and gas chromatography. Jornal of Agricultural and Food Chemistry. 48:5802–5807.

Takahashi M, Inai Y, Miyazawa N, Kurobayashi Y, Fujita A. 2013a. Key odorants in cured Madagascar vanilla beans (Vanilla planiforia) of differing bean quality. *Biosci. Biotechnol. Biochem.*77(3):606-611.

Takahashi M, Inay Y, Miyazawa N, Kurobayashi Y, Fujita A. 2013b. Identification of the Key Odorants in Tahitian Cured Vanilla Beans (*Vanilla tahitensis*) by GC-MS and an Aroma Extract Dilution Analysis. *Biosci. Biotechnol. Biochem.* 77(3)601-5.

Tokoro K, Kawahara S, Amano A, Kanisawa T, Indo M. 1990. Glucosides in vanilla beans and changes of their contents during maturation. Flavour science and Technology. Besseire Y, Thomas AF. John Wiley & Sons, Chichester, UK, pp. 73-76.

Van Dyk S, Holford P, Subedi P, Walsh K, Williams M, McGlasson WB. 2014. Determining the harvest maturity of vanilla beans. *Sci Hortic (Amsterdam)*. 168:249-257.

Van Dyk S, McGlasson WB, Williams M, Gair C.2010. Influence of curing procedures on sensory quality of vanilla beans. *Fruits*. 65(6):387-399.

Zhang S, Mueller C. 2012. Comparative analysis of volatiles in traditional cured Bourbon and Ugandan vanilla bean (Vanilla planifolia) extracts. *J Agric Food Chem*. 60(42):10433-10444.