DETERMINATION OF VANILLIN IN VANILLA (Vanilla planifolia Andrews) FROM LAMPUNG INDONESIA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

This paper describes a reverse-phase high performance liquid chromatographic (HPLC) method for the separation, identification and quantification of vanillin in ethanolic extracts of cured vanilla. The fresh green beans were cured by three methods: scalding in hot water, drying in the oven, and drying in the sun.

Two treatments for the cured beans before extraction, there were cutting cured vanilla about 2.5 cm and not cutting. The extraction was with Soxhletation and percolation method in 99.9 % ethanol. The vanillin was separated on C_{18} column using a mobile phase gradient of methanol - acidified water (10-90), detection at 280 nm. The HPLC technique allows a more accurate means of determining the vanillin content of vanilla than the spectrophotometric method .

Keywords: *Vanilla planifolia Andrews*, Vanillin, HPLC

INTRODUCTION

Vanilla plants belong to the orchid family, with more than 100 species known, but only three species, *Vanilla planifolia*, *V. tahitensis*, and *V. pompona,* are of practical relevance. By far the most important is *V. planifolia* [1]. Almost all vanilla imported into USA is *V. planifolia* Andrews grown on plantations in Madagascar, Indonesia, Reunion, Comores and the Seychelles. *V. tahitensis* Moore is grown in the French Pacific Island and exported primarily to France and Europe. The flavor profile of *V. tahitensis* Moore is totaly different to that of *V. planifolia* Andrews and is not favored by the food and beverage industries of the USA [2].

The main chemical constituent of vanilla is vanillin (4-hydroxy-3-metoxy benzaldehyde) [2,3], which was first isolated from vanilla by Gobley in 1858 [4]. The polar aromatic flavor compounds vanillin, ethyl vanillin, 4-hydroxy benzaldehyde, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol, vanillic acid, coumarin, piperonal, anisic acid and anisaldehyde are commonly found in extracts of natural and artificial vanilla flavor [2].

Among the many volatile aromatic compounds of vanilla extract, vanillin is the most characteristic component of the flavor. Several studies have indicated that glucovanillin and not vanillin are present in fresh vanilla pods [5]. Glucovanillin is hydrolyzed by endogenous β - glucosidase during the curing process to release vanillin [6].

Jurgens suggested that the ratio of the 4-hydroxybenzaldehyde to vanillin could be used to identify the geographical origin of vanilla extratcs as well as various forms of adulteration [3,7]. The ratio of 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde and vanillic acid to vanillin in natural vanilla extracts were used to confirm authenticity of vanilla extracts and as useful index for detecting the origin of vanilla extracts purchased in USA and UK [2,3,8]. Vanillin can be synthesized from readily available materials such as guaiacol (2-methoxyphenol) or eugenol (4-allyl-2-methoxy phenol) derived from clove oil [3].

Herrmann and Stockli reported a method for the control of vanilla products on the basis of identification and quantitation of 4-hydroxybenzyl alcohol, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, vanillyl alcohol, vanillin, ethyl vanillin and coumarin [9]. The flavor of vanilla extracts does very considerably, depending primarily upon the country of origin, but also upon crop year, curing techniques used, storage conditions, extraction method, and age of the vanilla extract it self [3].

HPLC column used in this study is C_{18} column as reported by Sujalmi [10]. Previous investigation on study efficiency of several HPLC columns in a vanillin analysis in a vanilla treatment suggested that C_{18} column is more efficient than CN column and phenyl Column.

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In this research the curing techniques used three methods, scalding in hot water, drying in the oven, and drying in the sun. Age of vanilla samples was seven months after pollinate, and extraction used Soxhletation and percolation method.

The objective of this work is to analyze vanillin in vanilla extract by HPLC method. A further objective is to compare the accuracy of the HPLC and ISO 5565:1982 spectrophotometric methods [11] in the determination of the vanillin content.

EXPERIMENTAL SECTION

Sample Preparation

Vanilla sampels from Kemiling, South Lampung about 1.5 kg were cured by three methods :

a. scalding in hot water at 65°C for 30 seconds (X_1).
b. drying in the oven at 60°C for 48 hours (X_2).
c. drying in the sun for 20 days and 2-3 hours every day (X_3).

Cured vanilla was fermented and dried in the wind, therefore the water content in the cured vanilla decreased from 55-65 % to 25-30 %.

The signs that vanilla had dried are:

a. vanilla colour was super black or brownish black, shine,
b. vanilla were deflected because water contain about 30 %.
c. vanilla had characteristic flavor, fragrant.

The last, cured vanilla were stored minimum for 2 months.

The result of cured vanilla were taken at random for pre-extraction:

a. weight the whole cured vanilla (Y_1) about 10 g for the Soxhletation extraction (Z_1) and 5 g for percolation (Z_2).
b. weight the cut cured vanilla (Y_2) 2.5 cm, about 10 g for the Soxhletation extraction (Z_1) and 5 g for the percolation (Z_2).

Extraction Procedures

Extract the cured vanilla of pre-extraction Z_1 in the Soxhlet extraction apparatus with 200 mL of the 99.9 % ethanol for 16 h. Transfer into a 250 mL volumetric flask. Rinse the flask of the extraction apparatus several times with small quantities of the 99.9 % ethanol, and pour the washings into the volumetric flask. Make up to the mark with the ethanol and mix well (ISO.5565-1982) [11].

Extract the cured vanilla of pre-extraction Z_2 in the percolation apparatus (Erlenmeyer). Submerge Z_2 about 35 mL of the 99.9 % ethanol in Erlenmeyer 100 mL for one night. Transfer into a 100 mL volumetric flask through paper filter. Rinse filter paper with 5 mL of the 99.9 % ethanol, store this paper filter for used at second filtering. Result of the cured vanilla from first submerge was pounded in mortar, then submerge again about 35 mL of the 99.9 % ethanol in Erlenmeyer for one night.

After submerge one night, ethanol from the second submerge was fused with ethanol from the first submerge through filtered used paper filter which used at first filtering. Rinse the cured vanilla with 99.9 % ethanol, and pour the washings into volumetric flask. Make up to the mark with the ethanol and mix well. (AOAC 19.011; 19.012) [12].

High Performance Liquid Chromatography (HPLC) – UV Spectrophotometric.

The vanillin was separated on a reverse – phase C_{18} Column using methanol-acidified water (10 + 90) and were detected at 280 nm. HPLC apparatus was Shimadzu and UV Spectrophotometric was Hitachi. The flow rate of HPLC is approximately 4 mL/min.

Preparation of Standard Solutions.

Standard solutions containing 0.5, 1.0, 2.0, 3.0 and 4.0 ppm of each standard were prepared in 99.9 % ethanol for HPLC. The solutions were stored at -2°C until required for analysis. Standard solutions for Spectrophotometric containing 1.0, 2.0, 3.0, 4.0 and 5.0 ppm of each standard were prepared in 99.9 % ethanol.

Quantification of Vanillin Content:

$$\text{Vanillin Content (\% w/w)} = \frac{C \times V}{W} \times 100\ \%$$

C = Concentration of standard solutions (ppm) from calibration curve at HPLC or Spectrophotometric method.
V = Initial volume of vanillin solution (L)
W = Sample weight (mg).

RESULTS AND DISCUSSION

Table 1 shows the linear regression equations and correlation coefficients (R) of the peak area measurement for vanillin standard in HPLC technique and of the absorbance in the ISO spectrophotometric assay method. Linear relations were observed for vanillin standard between 0 and 4 ppm with correlation coefficients of 0.997 at HPLC and between 0 and 5 ppm with correlation coefficients of 0.999 at UV spectrophotometric methods.

Vanillin content (% w/w) by the HPLC technique and the ISO spectrophotometric which the weight cured vanilla were 10 g for Soxhletation and 5 g for percolation, is shown in Table 2.

Table 1 The linear regression equations and correlation coefficients of the vanillin standard as determined by the HPLC technique and the ISO spectrophotometric assay method.

Technique / method	Intercept (a)	Slope (b)	Correlation coefficient (R)
HPLC	1880,91	20666,15	0,997
Spectrophotometric	-0,0029	0,1527	0,999

Table 2 Vanillin content (% w/w) from twelve treatments of vanilla samples determined by the HPLC technique and ISO spectrophotometric assay method

No	Treatment	Vanillin Content (% w/w)	
		By HPLC	By spectrophotometric method
1	$X_1 Y_1 Z_1$	2,12	2,48
2	$X_2 Y_1 Z_1$	2,75	3,15
3	$X_3 Y_1 Z_1$	2,45	2,71
4	$X_1 Y_2 Z_1$	3,56	3,89
5	$X_2 Y_2 Z_1$	3,20	3,72
6	$X_3 Y_2 Z_1$	1,82	2,20
7	$X_1 Y_1 Z_2$	1,22	1,39
8	$X_2 Y_1 Z_2$	1,12	1,30
9	$X_3 Y_1 Z_2$	1,14	1,41
10	$X_1 Y_2 Z_2$	2,02	2,44
11	$X_2 Y_2 Z_2$	2,28	2,86
12	$X_3 Y_2 Z_2$	2,14	2,46

Note :
X_1 = scalding in hot water
X_2 = drying in the oven
X_3 = drying in the sun
Y_1 = the whole cured vanilla
Y_2 = the cut cured vanilla
Z_1 = Soxhletation
Z_2 = percolation

Table 2 shows the amount (% w/w) of vanillin for twelve samples determined by the HPLC technique and the ISO spectrophotometric assay method. The data show that for the twelve vanilla samples analyzed, the spectrophotometric assay consistenly yielded higher values than the HPLC technique. The possible explanation for this, in the spectrophotometric assay method, vanillin is not pure (mixed with another substances) and absorbed at the same wavelength area. This will cause high absorption. Although HPLC uses detector UV but the sample goes through C18 column and it is separated before it comes to detector cell. This causes only vanillin which is detected and this also indicates relative smaller analytical result. However, the accuracy of this method is much better. The use of UV Spectrophotometric can be more accurate if a sample is separated firstly such as using special chromatography column. This way will obtain the same result with HPLC method with standard deviation less than 1 %. These present data are in agreement with previous data reported by Taylor [13], that the amounts of vanillin for four samples determined by ISO spectrophotometric assay yielded higher than by HPLC method.

Statistical analysis using variance analysis (ANOVA) shows that there is an influence among the treatment of (X1, X2, X3), a vanilla sample (Y1, Y2), and the extraction method of (Z1, Z2) upon vanillin content in the vanilla. This occurs in the HPLC method and also in the spectrophotometric method.

Investigation of the recovery process was carried out using HPLC analysis. Based on amount of required vanillin standard, the concentration of vanillin standard is 3.5 ppm. In the same condition of measurement, the analytical results using HPLC are 3.49, 3.51, 3.48, 3.48, 3.50, and 3.49 ppm respectively, with an average of 3.49 ppm. Therefore, it can be calculated that the recovery process obtained is 99.77 %. This result shows that the method used to determine the concentration of vanilla using HPLC is valid enough for the next measurement.

The objective of the vanilla curing with scalding in hot water, or drying in the oven, or drying in the sun was to stop vegetation growth and to chance enzim formed vanilla flavor. From Table 2, it can be seen that the highest vanillin content is at scalding in hot water, the cut cured vanilla and Soxhletation extraction (3.56 % by HPLC and 3.89 % by spectrophotometric method), then followed with drying in the oven (3.20 %, 3.72 %), and drying in the sun (1.82 % and 2.20 %).

CONCLUSION

The pre-extraction treatment for cured vanilla sliced about 2.5 cm resulted in higher vanillin content because there is direct contact perfectly between vanillin component in vanilla sample and extraction solvent.

The vanilin content resulted from the soxhletation extraction is higher than from the percolation extraction. This occurred because

there is continually solvent purity process in the soxhletation extraction. Therefore the density of the vanilin content in vanila extract is high.

The HPLC method offers a more accurate means of determining the vanillin content of vanilla than the ISO spectrophotometric assay, because by HPLC method vanillin was separated from other compounds.

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