Vanillin and Related Flavor Compounds in Vanilla Extracts Made from Beans of Various Global Origins

Arvind S. Ranadive

Premier Vanilla, Inc., East Brunswick, New Jersey 08816

Vanilla beans from seven different vanilla growing regions of the world were analyzed for vanillin, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, and vanillic acid. Beans were extracted to obtain singlefold extracts, and analysis was carried out using reversed-phase liquid chromatography. Analysis of the extracts, obtained from cured and uncured beans treated with β-glucosidase, indicates that all of the above-mentioned compounds are present in green beans as glycosides and are released upon curing. In addition to glycosides of these four known monophenols, there are at least three other major glycosides in green vanilla beans which are hydrolyzed during the curing process.

INTRODUCTION

The characteristic flavor and aroma of vanilla, developed in properly cured beans, is the result of a number of biochemical and chemical transformations. More than 170 volatile aromatic compounds have been identified in Madagascar beans (Klimes and Lamparsky, 1976). Of these, vanillin is the most abundant. Among the other major volatile constituents of vanilla aroma are *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, *p*-hydroxybenzyl alcohol, and vanillyl alcohol.

Guarino and Brown (1985) have developed a liquid chromatographic method that can be routinely and efficiently used for the quantitative analysis of vanillin, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, and vanillic acid. They analyzed 13 commercially available vanilla extracts and reported the quantitative data. Hermann and Stöckli (1982) reported a high-performance liquid chromatography (HPLC) method for the control of vanilla products through quantitative analysis of *p*-hydroxybenzyl alcohol, aldehyde, and acid, vanillin and vanillyl alcohol, ethyl vanillin, and coumarin. Wallace (1983) used an HPLC method to quantitatively analyze vanillin and seven other related phenolic compounds produced during the manufacture of vanillin from pulp mill effluent.

A correlation was shown to exist between vanillin and *p*-hydroxybenzaldehyde content of Madagascar vanilla by Jurgens (1981). He suggested the use of this ratio to identify the geographic origin of beans in an extract. Archer (1989) has suggested a similar correlation between vanillin and *p*-hydroxybenzoic acid and between vanillin and vanillic acid for genuine vanilla essences.

Vanillin, vanillyl alcohol, and one other aroma constituent of Bourbon vanilla were found to be present in their glycosidic forms in green beans by Goris (1947). Leong et al. (1989) found vanillin, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, and vanillic acid only in their glycosidic forms in ripe green Bourbon beans. Sagrero-Nieves and Schwartz (1988), on the other hand, showed the presence of significant levels of free vanillin and *p*-hydroxybenzaldehyde in maturing green Mexican beans.

The objective of this work was to collect quantitative data on the major aromatic volatiles in the cured vanilla beans from Madagascar, Indonesia, Mexico, Jamaica, Costa Rica, Tonga, and Tahiti. Except for Tahiti beans which belong to *Vanilla tahitensis* species, beans from all other world origins were of *Vanilla fragrans* (Salisbury) species, also known as *Vanilla planifolia* Andrews.

Another aim of this work was to confirm the presence of these aromatic volatile compounds as glycosides in green Jamaican beans and that they are released in their free form upon curing. The data obtained are presented in this paper.

EXPERIMENTAL PROCEDURES

Apparatus and Reagents. (*a*) *The liquid chromatograph* consisted of an Applied Biosystems Spectroflow 400 solvent delivery system, a Spectroflow 480 injector/valve module, and a Spectroflow 757 absorbance detector with UV detection set at 254 nm.

(*b*) *Column.* An Applied Biosystems Brownlee MPLC RP-8 Spheri-5 column (100 × 4.6 mm, catalog no. OS-MP)) packed with C_8 stationary phase, 5-μm particle size, was used in conjunction with a MPLC NewGuard cartridge (15 × 3.2 mm, catalog no. G08-013) containing C_8 stationary phase.

(*c*) *Solvents* used were LC grade methanol (J. T. Baker), LC grade water (Waters), 95% ethanol, and glacial acetic acid (Fisher Scientific).

(*d*) *The mobile phase* used was methanol–acidified water (10 + 90) pumped at 1.5 mL/min. Water was acidified by adding 10 mL of glacial acetic acid/800 mL.

(*e*) *Sample filters* used were 0.45-μm nylon 66 membrane alcohol-compatible filters (Rainin).

(*f*) *Standards.* Vanillin (Sigma), *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, and vanillic acid were obtained from Kodak. *p*-Hydroxybenzyl alcohol and vanillyl alcohol were obtained from Aldrich. Caution should be exercised while using these chemical irritants.

Standard Preparation. Vanillin (1.2000 g), vanillic acid (0.0800 g), *p*-hydroxybenzoic acid (0.0200 g), and *p*-hydroxybenzaldehyde (0.0600 g) were weighed into a 100-mL volumetric flask and diluted to volume with 95% ethanol. A 10-mL aliquot was further diluted to 100 mL with 40% ethanol and filtered through 0.45-μm nylon 66 filter prior to injection into chromatograph.

Extract Preparation. Approximately 100 g of cured vanilla pods was finely chopped without crushing. A 10-g sample of the chopped beans was used for extraction with 75 mL of 44% aqueous ethanol for 48 h at 45 °C. The mixture was occasionally stirred during this time. The mixture was then filtered and the filter cake pressed and washed with 36% ethanol until the total volume of filtrate and washings was 100 mL (this is singlefold vanilla extract).

Enzyme Treatments. *Uncured beans.* Jamaican green vanilla beans were provided by Elan, Inc., Newark, NJ, and were kept refrigerated after harvest until used for these experiments. One hundred grams of green beans was immersed in a boiling water bath for 5 min to inactivate all native enzymes. After cooling to ambient temperature, the beans were pureed in a Waring Blendor and separated into two equal portions. To one

0021-8561/92/1440-1922$03.00/0

Table I. Important Flavoring Components of Cured Vanilla Beans of Different Geographic Origins (Milligrams per 100 mL of Singlefold Extract)

bean source	p-hydroxybenzoic acid	p-hydroxybenzaldehyde	vanillic acid	vanillin HPLC method	vanillin UV method
Madagascar	5.6	13.7	15.0	164.0	184.0
Indonesia	3.4	9.3	7.7	117.0	131.0
Mexico	4.0	7.0	13.0	90.0	100.0
Costa Rica	5.2	14.0	12.0	135.0	161.0
Jamaica	n.d.	8.4	4.2	216.0	265.0
Tonga	2.1	10.0	7.6	197.0	320.0
Tahiti	32.8	13.0	4.4	103.0	120.0
commercial extract	43.8				
1	2.2	8.5	7.5	133.0	
2	2.0	10.6	8.3	154.0	
3	1.5	9.2	6.8	133.0	

portion was added 20 mL of β-glucosidase solution (0.25 mg of enzyme/mL) to obtain an approximate concentration of 0.5 mg of enzyme/g of dry bean weight, followed by thorough mixing and incubation at 40 °C for 1 h. The mixture was then dried to a moisture content of less than 5%. The second portion was treated similarly except 20 mL of water containing no enzyme was added to the puree. Both the enzyme-treated and control dry beans were then extracted as described under Extract Preparation to obtain singlefold extracts.

Cured Beans. Madagascar, Tonga, Jamaica, and Tahiti beans were chopped to about 1/4-in. pieces. To 20 g of each of the chopped beans was added 25 mL of β-glucosidase solution (0.25 mg of enzyme/mL) to obtain an approximate concentration of 0.5 mg of enzyme/g of dry bean weight, and the beans were incubated at 40 °C for 1 h. The enzyme-treated beans were then extracted with aqueous ethanol to obtain singlefold extracts. Singlefold extracts were also prepared from the beans not treated with enzyme.

Sample Preparation and Calculations. A 10-fold dilution of the singlefold extract, containing less than 0.3 g of vanillin/100 mL of extract, was made using 40% aqueous ethanol. The samples were filtered through 0.45-μm nylon 66 filters. Concentrations of the major components in the samples were calculated by peak area proportioning using external standards.

RESULTS AND DISCUSSION

Quantitative data on vanillin, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, and vanillic acid present in singlefold extracts made from cured vanilla beans of different geographic origins are presented in Table I. Both p-hydroxybenzyl alcohol and vanillyl alcohol were also found to be present in all extracts, but, due to their inadequate separation from other peaks in our method, it was not possible to obtain quantitative data on these compounds. Although only one batch of beans per geographic source was used, representative samples from large batches (1–3 tons) of beans from each geographic area were used for this analysis. Also, reproducibility of the extraction procedure was ascertained by replicate extractions of Madagascar beans. It should be mentioned, however, that the results obtained from only one sample per bean origin allow the most general conclusions to be drawn.

Beans of *V. fragrans* species of different geographic origins showed little variation in p-hydroxybenzoic acid and p-hydroxybenzaldehyde content. Their vanillin and vanillic acid contents, however, showed considerable variation. It is known that the bean maturity at harvest and the manner in which the beans are cured have considerable effect on their vanillin content (Ranadive et al., 1983). Since the Tonga, Jamaica, and Indonesia beans showed the vanillin content of fully matured adequately cured beans, the presence of lower vanillic acid in these beans may be related to their geographic origin. Smaller variations in p-hydroxybenzoic acid and p-hydroxybenzaldehyde contents in the beans which showed considerable variation in their vanillin and vanillic acid content indicate that the maturity, geographic origin, or curing processes may have little effect on their formation. Overall lower values for all four parameters for the three commercial extracts may be the reflection of inadequate amounts of beans used for their preparation.

Table II. Ratios of Vanillin to Other Important Flavoring Components

bean source	ratio of vanillin to p-hydroxybenzoic acid	ratio of vanillin to p-hydroxybenzaldehyde	ratio of vanillin to vanillic acid
Madagascar	29.3	12.0	10.9
Indonesia	34.4	12.6	15.2
Mexico	22.5	12.9	6.9
Costa Rica	26.0	9.6	11.3
Jamaica	n.d.	25.7	51.4
Tonga	93.8	19.7	25.9
Tahiti	2.4	7.9	23.4

The most significant difference between *V. fragrans* and *V. tahitensis* beans was seen in their p-hydroxybenzoic acid content, which appears to be about 10 times higher in *V. tahitensis* beans. *V. tahitensis* beans also contain anisic acid, anisic aldehyde, and heliotropin, all of which are not detected in *V. fragrans* beans.

The ratios of vanillin to p-hydroxybenzoic acid, vanillin to p-hydroxybenzaldehyde, and vanillin to vanillic acid are given in Table II. The ratio of vanillin to p-hydroxybenzaldehyde has been used to authenticate vanilla extracts, and its range is reported to be 10.9–18.4 (Jurgens, 1981). This ratio for Madagascar, Indonesia, Mexico, Costa Rica, and Tonga beans appears to be consistent with the range reported by Jurgens. As can be seen from the data in Table II, other ratios did not show any particular trend. Archer (1989), working on commercial and laboratory-prepared Bourbon and Java vanilla extracts, also observed much larger variation in the vanillin to p-hydroxybenzoic acid and vanillin to vanillic acid ratios than in the vanillin to p-hydroxybenzaldehyde ratio. It is not surprising to see the inconstancy in the ratios for beans of different regions since their vanillin content, which is dependent on bean maturity at harvest, environmental factors, and curing practices, showed large variation.

The data on Jamaica vanilla beans, green and after curing, presented in Table III show that all four important flavoring components increased after the beans were cured. The same components increased dramatically when green beans whose native enzymes were heat inactivated were treated with added β-glucosidase. The results indicate that these four flavoring components of vanilla are present in green beans in their glycosidic forms and are produced in their free form by the native glycosidases during curing. It is interesting to see that the beans treated with added enzyme produced higher amounts of these components compared to beans cured normally (with native enzymes system intact and no added enzymes). This may be due

Table III. Determination of Selected Important Flavoring Components of Green, Cured, and Enzyme-Treated Killed Green Jamaica Vanilla Beans (Milligrams per 100 mL of Singlefold Equivalent Extract)

bean source	*p*-hydroxybenzoic acid	*p*-hydroxybenzaldehyde	vanillic acid	vanillin HPLC method
Jamaican green bean extract	0.00	3.5	0.3	40.0
Jamaica lab-cured bean extract	1.2	11.1	4.8	240.0
Jamaica killed green bean extract after external β-glucosidase treatment	2.8	22.5	8.9	370.0

Table IV. Effect of β-Glucosidase Treatment of Cured Vanilla Beans on the Vanillin Content

	vanillin, mg/100 mL of extract	
bean source	control	treated with β-glucosidase
Madagascar	171	182
Tonga	147	165
Jamaica	229	285
Tahiti	80	80

to (a) inadequate amounts of native enzymes present in green beans, (b) inadequate amounts of enzyme–substrate contact, or (c) poisoning of the native enzymes by oxidized phenols produced simultaneously during curing. This oxidation of phenols was (unintentionally) kept to a minimum in the enzyme-treated beans in our experiments, which helped added enzyme to complete its process more effectively.

If the native vanilla bean enzymes do not complete the hydrolysis of vanilla glycosides during normal curing, then the commercially cured beans should produce more vanillin if treated with external enzyme prior to extraction than the nontreated beans. To test this hypothesis, commercially cured Madagascar, Tonga, Jamaica, and Tahiti beans were treated with β-glucosidase dissolved in water 1 h prior to extraction with aqueous ethanol. Control treatments were carried out without the enzyme. The results in Table IV indicate that commercially cured Madagascar, Tonga, and Jamaica beans did produce increased amounts of vanillin upon external enzyme treatment. This strengthens our observations that the hydrolysis of glycosides is not completely accomplished in the normal curing process. Tahiti beans did not show increase in the vanillin content upon enzyme treatment, probably due to complete hydrolysis of glucovanillin during commercial curing. This could also have happened if the added enzyme was inhibited by Tahiti bean vanilla phenolics.

ACKNOWLEDGMENT

I thank Elan, Inc., of Newark, NJ, for use of their laboratory facility. I am grateful to Mr. George Kapp of Elan, Inc., for providing Jamaican green vanilla beans used in this work.

LITERATURE CITED

Archer, A. W. Analysis of vanilla essences by high-performance liquid chromatography. *J. Chromatogr.* **1989**, *462*, 461.

Goris, M. A. Formation du parfum de la vanille. *Ind. Parfum.* **1947**, *2*, 4.

Guarino, P. A.; Brown, S. M. Liquid Chromatographic Determination of Vanillin and Related Flavor Compounds in Vanilla Extract: Cooperative Study. *J. Assoc. Off. Anal. Chem.* **1985**, *68*, 1198.

Herrmann, A.; Stöckli M. Rapid control of vanilla-containing products using high-performance liquid chromatography. *J. Chromatogr.* **1982**, *246*, 313.

Jurgens, U. The vanillin/p-hydroxybenzaldehyde ratio in bourbon vanilla. *Lebensmittelchem. Gerichtl. Chem.* **1981**, *35* (5), 97.

Klimes, I.; Lamparsky, D. Vanilla Volatiles—A Comprehensive Analysis. *Int. Flavours Food Addit.* **1976**, Nov/Dec, 272.

Leong, G.; Archavlis, A.; Derbesy, M. Research on the Glucoside Fraction of the Vanilla Bean. *J. Essent. Oil Res.* **1989**, *1*, 33.

Ranadive, A. S.; Szkutnica, K.; Guerrera, J. G.; Frenkel, C. Vanillin Biosynthesis in Vanilla Beans. *Proceedings of the 9th International Congress of Essential Oils, Singapore*; Allured Publishing: Wheaton, IL, 1983; Book 2, p 147.

Sagrero-Nieves, L.; Schwartz, S. J. Phenolic Contents of Vanilla planifolia as Affected by Harvest Period. *J. Food Compos. Anal.* **1988**, *1*, 362.

Wallace, E. M. HPLC of Vanillin and Related Phenols. *J. Chromatogr. Sci.* **1983**, *21*, 139.

Received for review March 30, 1992. Revised manuscript received July 8, 1992. Accepted July 20, 1992.

Registry No. Vanillin, 121-33-5; *p*-hydroxybenzoic acid, 99-96-7; *p*-hydroxybenzaldehyde, 123-08-0; vanillic acid, 121-34-6; *p*-hydroxybenzyl alcohol, 623-05-2; vanillyl alcohol, 498-00-0; β-glucosidase, 9001-22-3; anisic acid, 1335-08-6; anisic aldehyde, 50984-52-6; heliotropin, 120-57-0.