Determination of Glucovanillin and Vanillin in Cured Vanilla Pods

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Glucovanillin purified from cured vanilla beans and commercial vanillin were used to develop a reversed-phase HPLC method that enables the separation, identification, and quantification of both glucovanillin and vanillin extracted from cured Java and Bourbon vanilla beans. The HPLC procedure was used to evaluate the potential of various methods for the simultaneous extraction of glucovanillin and vanillin from cured beans. Satisfactory results were obtained for glucovanillin extracted with a 24-h Soxhlet extraction in 47.5% ethanol and for vanillin with a 24-h extraction by maceration in 47.5% ethanol or 80% methanol. None of the tested methods correctly met the requirement for a quantitative and simultaneous extraction of both compounds. The slight underestimation of vanillin content by Soxhlet extraction could probably be overcome by modifying the extraction parameters.

Keywords: *Vanilla; vanillin; glucovanillin; HPLC determination; extraction method*

INTRODUCTION

Natural vanilla is extracted from cured beans of *Vanilla fragrans*. Among the many volatile aromatic compounds of vanilla extract, vanillin is the single most characteristic component of the flavor. Several studies have indicated that glucovanillin and not vanillin is present in fresh vanilla pods (Goris, 1924; Arana, 1943; Leong et al., 1989a). Glucovanillin is hydrolyzed by endogenous β-glucosidase during the curing process to release vanillin (Arana, 1943).

Traditional curing, an empirical technique, fails to completely hydrolyze vanilla glucosides. Ranadive (1992) showed that cured Tahiti, Tonga, Jamaica, and Madagascar beans produced more vanillin upon exogenous β-glucosidase treatment, with a yield improvement reaching up to 24%. Recently, vanilla extract prepared on a pilot scale was reported to have a vanillin content increase of up to 14% after treatment of cured beans with exogenous pectinase and β-glucosidase (Mane and Zuccha, 1993).

To date, vanillin content and bean humidity are among the important factors used to assess potential vanillin yield of different cured vanilla bean lots (Gillette and Hoffman, 1992). However, the traditional vanilla extraction process could be modified in the near future to take advantage of the latest development in residual glucovanillin enzymatic hydrolysis and improve vanillin recovery from cured beans. Therefore, glucovanillin content of cured beans could become an important factor for determining vanillin yields from various bean lots.

Determination of vanillin in vanilla extract by HPLC is reported in the literature (Guarino and Brown, 1985; Ranadive, 1992; Taylor, 1993; Lamprecht et al., 1994), but few papers describe works on quantitative glucovanillin determination. High-performance liquid chromatography (HPLC) methods were used to estimate vanilla glucosides from green beans (Tokoro et al., 1990; Leong et al., 1989a). Measurement of reducing sugars liberated from glucovanillin hydrolysis was also used to indirectly assess glucovanillin in beans during curing (Arana, 1943). To our knowledge, no study has dealt with the direct determination of glucovanillin in cured vanilla beans. In this work, we present a HPLC method to quantify both glucovanillin and vanillin from vanilla extract. We have also investigated different ways to simultaneously extract both compounds from cured vanilla beans.

MATERIALS AND METHODS

Plant Material. Cured vanilla pods were provided by Givaudan-Roure (Brampton, Ontario, Canada) in a chopped form (1–2-cm pieces). Before extraction, chopped vanilla beans were ground in a centrifuge grinding mill (type ZM-1) fitted with a stainless steel sieve of 1 mm (Retsch, Haan, Germany).

Chemicals. All reagents used for chromatography were of HPLC grade. Ethanol (95%) used for extraction was of food grade. All other solvents were of analytical grade or higher. Vanillin, vanillic acid, and almond β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21; 9.6 U/mg) were purchased from Sigma Chemical Co. (St. Louis, MO) and ethylvanillin, *p*-hydroxybenzoic acid, and *p*-hydroxybenzaldehyde from Omega Chemical Co. (Québec, Canada).

Isolation of Glucovanillin. *(A) Extraction.* A total of 112 g of ground Java beans were extracted in eight Erlenmeyer flasks by adding 700 mL of 50% ethanol to each flask and incubating at 63 °C for 2 h under orbital agitation (200 rpm). After filtration on Whatman No. 2 paper, each resulting cake was reextracted with 350 mL of 50% ethanol, and the extract was filtrated as just described. The vanilla extract was then concentrated on a rotary evaporator to a final volume of 500 mL and washed four times with 500 mL of diethyl ether. After addition of one part of 95% ethanol to one part of concentrate, the resulting precipitate was removed by filtration on Whatman No. 2 paper. The filtrate was further purified by batch processing on 35 g of C-18 silica gel (30–70 μm; Mandel Scientific Co., Guelph, Canada) retained in a funnel fitted with a fritted glass disk and fixed on a filtering flask. Twenty milliliters of filtrate was added at once to the gel that had previously been washed with methanol and water. After a 5-min period of standing, vacuum was applied, and the gel was rinsed with 250 mL of water before elution of glucovanillin with 150 mL of methanol. Silica gel was regenerated with

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250 mL of water before starting a new purification cycle. All methanol fractions were pooled, evaporated on a rotary evaporator, solubilized in 40 mL of 47.5% ethanol, and stored at 4 °C.

(B) Purification. Preparative HPLC was carried out on a C-18 reversed-phase column (PrepPack μBondapack cartridge; 25 × 100 mm; 10 μm) that was preceded by a guard cartridge (25 × 10 mm) and maintained in a RCM 25 × 10 cartridge module (Waters Chromatography, Milford, MA). The HPLC chromatograph (System Gold, solvent module 126; Beckman, San Ramon, CA) was equipped with a 3-mL sample loop, an injection valve from Rheodyne (Berkeley, CA), and a UV detector (System Gold, detector module 166; Beckman). The mobile phase was composed of water acidified with 1.25% acetic acid and methanol in a ratio 90:10 (v/v). Elution was isocratic, with a flow rate of 8 mL/min, and the effluent was monitored at 270 nm. The peak corresponding to glucovanillin was collected between 29.5 and 31.5 min. A 15-min wash in methanol completed the chromatographic cycle.

The glucovanillin peak in preparative HPLC was first tentatively identified from an enzymatic release of vanillin from glucovanillin: collected fractions were evaporated on a rotary evaporator, resuspended in 1 mL of acetate buffer (0.1 M, pH 5), and almond β-glucosidase was added to obtain a concentration of 1 U/mL. Samples were incubated for 1 h at 37 °C with reduced agitation, and the reaction was stopped by addition of 1 mL of ethanol. Vanillin was detected by analytical HPLC. Thin-layer chromatography of the various HPLC fractions was also carried out on silica gel 60 plates to detect glucovanillin, as described by Leong et al. (1989b).

All collected glucovanillin fractions obtained from preparative HPLC were pooled and evaporated on a rotary evaporator. Finally, glucovanillin was solubilized in methanol and filtered through a 0.45-μm filter to give the stock solution.

Identification of Glucovanillin. *(A) UV Spectroscopy.* Samples of glucovanillin stock solution were evaporated under nitrogen flow and resuspended in ethanol. A UV scan was obtained between 190 and 350 nm on a Beckman DU-7 spectrophotometer.

(B) NMR Study. ^{1}H and ^{13}C NMR spectra were obtained from glucovanillin in deuterated methanol on a Bruker AM-300 wide-bore NMR spectrometer. The ^{1}H spectra were run at 300.13 MHz in a 5-mm dual $^{1}H/^{19}F$ probe at 25 °C, and ^{13}C spectra were run at 75.468 MHz in a 10-mm broad-band probe at 27 °C. The references for the chemical shift positions were residual protonated methanol at 3.30 ppm for ^{1}H and deuterated methanol at 49.0 ppm for ^{13}C. The ^{1}H chemical shift positions and coupling constants for all coupled signals were determined by spectral simulation with PANIC (Bruker Spectrospin) and MLDC8 (Quantum Chemistry Program Exchange 100, Indiana University). The standard deviations obtained for coupling constants were 0.05 Hz for the vanillin moiety and 0.2 Hz for the sugar moiety. Assignments of the glucose ^{1}H chemical shifts were determined by the 1D HOHAHA experiment (Davis and Bax, 1987) with increasing mixing times from the anomeric proton. The position of the substituents on the aromatic ring was confirmed by ^{1}H NOE difference experiments indicating medium enhancements between H-7 and H-3, H-8 and H-3, H-1′ and H-6, and a weak enhancement between H-8 and H-5. The ^{13}C chemical shift positions were assigned from 2D HETCOR and HMBC spectra.

(C) Mass Spectrometry. Glucovanillin was analyzed using static probe liquid secondary ion mass spectrometry (LSIMS) with a Fisons 70-250 SEQ instrument. About 1 μg of dried glucovanillin was dissolved in 5 μL of glycerol on the probe tip. The spectrum was recorded by scanning from *m/z* 600 to 60.

Quantification of Glucovanillin. Glucovanillin concentration in the stock solution was determined by measuring the vanillin released from glucovanillin by β-glucosidase. The determination was repeated four times. After evaporating the solvent from a sample of the glucovanillin stock solution (500–600 μg of glucovanillin) under a nitrogen flow, 750 μL of a β-glucosidase solution containing acetate buffer (0.1 M, pH 5) at 9.5 U/mL was added. This mixture was then incubated for 2 h at 37 °C with reduced agitation. The reaction was stopped by adding 600 μL of 95% ethanol, and 150 μL of ethyl vanillin in ethanol (4 mg/mL) was added as internal standard. After filtration through a 0.45-μm filter, vanillin content was determined by analytical HPLC, using commercial vanillin as a standard, and the glucovanillin content was calculated. A multicomponent calibration table (six levels in triplicate) was built with commercial vanillin (0.1–5 μg/injection), glucovanillin from the stock solution (0.1–1.5 μg/injection), and ethyl vanillin (internal standard) under the analytical HPLC conditions described later and with the UV detector set at 270 nm (i.e., the glucovanillin λ_{max}).

Table 1. Methods Used for Vanilla Extractions

method	extraction	solvent	%
1	Soxhlet (control)	ethanol	47.5
2a	homogenization	ethanol	95
2b		ethanol	47.5
2c		methanol	100
2d		methanol	80
3a	5 min in boiling water + homogenization	ethanol	47.5
3b	20 min in boiling water + homogenization	ethanol	47.5
3c	5 min in boiling water + homogenization	methanol	80
3d	20 min in boiling water + homogenization	methanol	80
4a	maceration 24 h	ethanol	47.5
4b		methanol	80

Analytical HPLC. Glucovanillin and vanillin were fractionated on a C-18 reversed-phase column (ODS2-Spherisorb, 10 μm, 25 × 0.46 cm; Chromatography Sciences Co., Montréal, Canada). The HPLC system was composed of a series 410 LC Bio pump, an automatic ISS2 sample processor set for 10-μL injection, and a LC-235 diode array detector from Perkin-Elmer (Norwalk, CT). Solvent A was water acidified with 1.25% acetic acid (v/v) and solvent B was methanol. An elution program was developed to separate glucovanillin, vanillin, and ethyl vanillin from other vanilla extractable compounds. Final analysis conditions were as follows for a flow rate of 1 mL/min: linear gradient of solvent A from 95 to 90% over a period of 5 min; hold for 5 min; linear gradient to 65% solvent A over a period of 30 min; hold for 10 min. Each run was completed by washing with 100% solvent B for 15 min and reconditioning with 95% solvent A for 10 min.

Simultaneous Extraction of Glucovanillin and Vanillin. The different extraction methods of Java and Bourbon type vanilla are summarized in Table 1. Four experimental procedures were used.

(A) Soxhlet (Method 1). Two grams of vanilla was extracted with 200 mL of 47.5% ethanol for 24 h in a Soxhlet apparatus heated at 200 °C. After ethylvanillin was added as the internal standard, the extract was transferred to a volumetric flask, adjusted to 250 mL with ethanol 47.5%, and concentrated 2.5-fold on a rotary evaporator before HPLC analysis.

(B) Homogenization (Method 2). An Ultra-Turrax apparatus equipped with a S25N-10G rotor (Janke and Kunkel GmbH, Hohenstaufen, Germany) was used for vanilla extraction. After 6 mL of solvent was added to a vial containing 0.2 g of vanilla and internal standard, three 1-min bursts of homogenization (20 000 rpm) separated by 1 min of standing on ice were applied. The homogenate was then filtrated on a Whatman No. 2 paper and rinsed three times with 1 mL of solvent. Extracts were brought to a volume of 10 mL with the same solvent, filtered on a 0.45-μm filter, and analyzed by HPLC.

(C) Heat Treatment (Method 3). Six milliliters of solvent was added with a suitable amount of internal standard to screw-capped tubes containing 0.2 g of vanilla. The tightly closed tubes were heated for up to 20 min in boiling water and chilled in cold water. Extraction was performed as described for method 2.

(D) Maceration (Method 4). Vanilla (0.2 g) was macerated for 24 h with orbital agitation (150 rpm) at 60 °C in 9 mL of

Table 2. NMR Chemical Shift and Coupling Data for Glucovanillin

position	^{13}C chemical shift (ppm)	^{1}H chemical shift (ppm)	$^{1}H-^{1}H$ coupling constant (Hz)
glucose moiety			
1′	101.81	5.065	$J_{1'-2'} = 7.9$
2′	74.71	3.539	$J_{2'-3'} = 9.5$
3′	78.39	3.484	$J_{3'-4'} = 9.6$
4′	71.23	3.401	$J_{4'-5'} = 7.8$
5′	77.87	3.481	$J_{5'-6'} = 5.7$
			$J_{5'-6''} = 2.3$
6′	62.44	3.689	$J_{6'-6''} = 12.1$
6″		3.883	
vanillin moiety			
1	153.51		
2	151.29		
3	111.82	7.492	$J_{3-5} = 1.8$
4	132.86		
5	126.94	7.513	$J_{5-6} = 8.4$
6	116.57	7.307	
7	56.65	3.912	
8	192.99	9.829	

solvent containing an internal standard. After filtration on a Whatman No. 2 paper, the cake was rinsed with a small portion of solvent and the filtrate was brought to a final volume of 10 mL with the same solvent.

For each vanilla type, an analysis of variance was performed on vanillin and glucovanillin extraction yields. Means were compared when necessary by the Tukey–Kramer multiple comparison test.

RESULTS AND DISCUSSION

Glucovanillin Identification. Glucovanillin purified from cured vanilla beans was clearly identified. Maximal UV absorption peaks at 270 and 305 nm (not shown) correspond to the values reported for glucovanillin from green vanilla beans (Leong et al., 1989a) or obtained synthetically (Leong et al., 1989b). LSIMS spectroscopy gave a *m/z* ratio of 315 (M + H), confirming the glucovanillin molecular weight. The fragment corresponding to vanillin was also observed (M + H = 153).

Glucovanillin identification was confirmed by the NMR study. The NMR data are shown in Table 2. Assignments for carbons are in agreement with those reported for glucovanillin isolated from green vanilla beans (Leong et al., 1989a,b). We have included detailed assignments for the protons and provided the proton–proton coupling constants that were not reported by Leong et al. (1989a,b).

HPLC Determination of Glucovanillin and Vanillin. Quantification of glucovanillin from the stock solution by spectrophotometry at 270 nm using a molar absorption coefficient of 11 300 (Leong et al., 1989a) was inappropriate because a minor contaminant absorbing at the same wavelength was detected by analytical HPLC. Enzymatic conversion of glucovanillin to vanillin was therefore necessary to obtain an accurate determination of glucovanillin concentration. Glucovanillin was completely converted to vanillin by β-glucosidase and no peak other than vanillin was generated from the enzymatic hydrolysis. The glucovanillin contaminant was not hydrolyzed by the enzyme. Glucovanillin concentration in stock solution was 322 μg/mL.

The HPLC technique used to analyze vanilla extracts was optimized to resolve glucovanillin and vanillin from other vanilla components (Figure 1). Vanillic acid, *p*-hydroxybenzoic acid, and *p*-hydroxybenzaldehyde were also well separated. Although we did not test the HPLC method with extract from green vanilla beans, it is likely

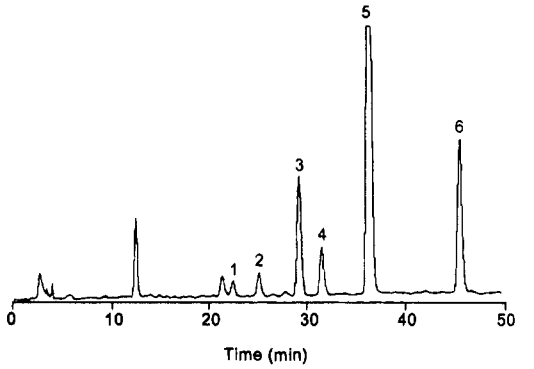

Figure 1. Reversed-phase HPLC of a vanilla extract from cured Bourbon beans: (1) glucovanillin; (2) *p*-hydroxybenzoic acid; (3) *p*-hydroxybenzaldehyde; (4) vanillic acid; (5) vanillin; and (6) ethyl vanillin.

that quantification of vanillin and glucovanillin in these extracts is achievable without modification. Under the chromatographic conditions used, many hundreds of 10-μL injections were performed without any loss of resolution. The following relationship was used for the determination of glucovanillin and vanillin:

$$\text{weight } x/\text{weight IS} = f_x(\text{area } x/\text{area IS}) \quad (1)$$

f_x is the response factor of the component "*x*" relative to the internal standard, "*x*" is vanillin or glucovanillin, and "IS" is the internal standard (i.e., ethylvanillin). The response factors relative to ethylvanillin obtained from linear regressions were 1.4531 ($r = 0.999$) and 0.9740 ($r = 0.999$) for glucovanillin and vanillin, respectively. The coefficient of variation of the chromatographic method was <2%.

Glucovanillin is not commercially available, so we suggest recalibration of glucovanillin, when necessary, with the response factor of glucovanillin relative to vanillin (*F*).

$$F = f_{(\text{glucovanillin})}/f_{(\text{vanillin})} \quad (2)$$

Therefore, by injecting a calibration mixture containing only ethylvanillin and vanillin, the response factor of glucovanillin relative to the internal standard "$f_{(\text{glucovanillin})}$" is obtained by using $f_{(\text{vanillin})}$ and *F*.

Glucovanillin and Vanillin Extraction Procedures. Glucovanillin and vanillin extraction yields of Java and Bourbon vanilla beans by various procedures are presented in Table 3. Only the most efficient methods, as determined from the extraction of Java beans, were used with Bourbon beans. Soxhlet extraction was chosen as the control method. For Java bean, ~10% more vanillin was recovered after an extraction by homogenization when 47.5% ethanol (extraction method 2b) or 80% methanol (extraction method 2d) were used instead of 95% ethanol (extraction method 2a) and 100% methanol (extraction method 2c), respectively. The same effect was also observed for glucovanillin, especially with ethanol extraction, where glucovanillin yield increased by about 2-fold when 47.5% ethanol was used. The higher polarity of solvents containing more water facilitated the solubilization of vanillin and particularly that of glucovanillin. Therefore, 80% methanol and 47.5% ethanol were used for subsequent extraction studies.

Use of heat pretreatment was also successful in improving vanillin extraction. When ethanolic extraction (extraction method 2b) was preceded by 5- and 20-

Table 3. Extraction of Vanillin and Glucovanillin from Vanilla Cured Beans

	Java		Bourbon	
extraction method	vanillin[a] (%)	glucovanillin (%)	vanillin (%)	glucovanillin (%)
1	100.0 (7.0)	100.0 (12.1)	100.0 (1.5)	100.0 (10.2)
2a	61.4 (1.3)	32.6 (4.2)		
2b	73.8 (3.0)	72.1 (9.2)		
2c	69.1 (2.6)	59.6 (9.8)		
2d	78.4 (2.9)	72.4 (7.5)		
3a	84.9 (0.9)	61.1 (3.0)		
3b	100.1 (1.6)	68.0 (8.3)	95.9 (1.5)	88.5 (4.6)
3c	88.3 (1.4)	75.0 (6.2)		
3d	98.5 (2.6)	71.5 (11.1)	97.2 (2.1)	80.3 (4.8)
4a	107.5 (2.0)	74.8 (6.4)	101.1 (1.9)	77.4 (4.5)
4b	108.7 (1.7)	78.3 (10.9)	102.1 (1.8)	80.0 (4.0)

[a] Percentage relative to method 1 (Soxhlet); mean ± SD for 3–6 replications.

min heat treatments (extraction methods 3a and 3b), 10.1 and 26.2% more vanillin respectively, was recovered. Glucovanillin yield was, however, not improved by such pretreatments. The same effect on vanillin yield was observed with the 80% methanol extraction (extraction method 2d); that is, vanillin relative recovery was 9.9 and 20.1% higher after a 5- and 20-min heating periods, respectively (extraction methods 3c and 3d). Heat treatment of Bourbon beans resulted in a better glucovanillin extraction in 47.5% ethanol (extraction method 3b) than in 80% methanol (extraction method 3d), but the difference was not statistically significant ($P < 0.05$). No significant difference between the same treatments was detected for Java beans.

Bean maceration in 50% ethanol (extraction method 4a) or 80% methanol (extraction method 4b) was very efficient in extracting vanillin. Extraction yields were slightly superior ($P < 0.01$) or similar to those obtained with a Soxhlet extraction for Java beans and for Bourbon beans, respectively. Unfortunately, bean macerations did not allow extraction of >80% of the amount of glucovanillin obtained with Soxhlet extractions. Therefore, that approach is suitable when a good extraction of vanillin alone is required. Solvent mixtures containing methanol:ethanol:water (40:40:20 or 35:35:30, v/v/v) were also tested (not shown), but did not improve the glucovanillin or vanillin extractions.

Among the many extraction procedures used, Soxhlet extraction (extraction method 1) was the most efficient method to simultaneously extract vanillin and glucovanillin from Java and Bourbon beans. The highest glucovanillin yield was obtained with this procedure for both types of vanilla beans, but vanillin was slightly underestimated compared with the yields obtained with the maceration procedure for Java beans powder.

Conclusion. Vanillin and glucovanillin content of cured vanilla bean extracts can be easily determined by the HPLC procedure developed in this work. Simultaneous determination of both compounds in cured beans is limited by the difficulty in obtaining a quantitative extraction of vanillin and glucovanillin. Satisfactory results are obtained for glucovanillin with Soxhlet extractions and for vanillin with maceration procedures. Optimization of Soxhlet or maceration procedures would be indicated to fill the requirement for a good extraction of both glucovanillin and vanillin in cured vanilla beans and therefore take full advantage of the HPLC method presented in this work.

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LITERATURE CITED

Arana, F. Action of a β-Glucosidase in the curing of vanilla. *Food Res.* **1943**, *8*, 343–351.

Davis, D. G.; Bax, A. Simplification of ^{1}H NMR spectra by selective excitation of experimental subspectra. *J. Am. Chem. Soc.* **1987**, *107*, 7197–7198.

Gillette, M. H.; Hoffman, P. B. In *Encyclopedia of Food Science and Technology*; Hui, H. Y., Ed.; Wiley: New York, 1992; Vol. 4, pp 2641–2657.

Goris, A. On the chemical composition of green vanilla fruits and the process of development of vanilla aroma. *Compt. Rend.* **1924**, *170*, 70–72.

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*, 1198–1201.

Lamprecht, G.; Pichlmayer, F.; Schmid, E. R. Determination of the authenticity of vanilla extracts by stable isotope ratio analysis and component analysis by HPLC. *J. Agric. Food Chem.* **1994**, *42*, 1722–1727.

Leong, G.; Archavlis, A.; Derbesy, M. Research on the glucoside fraction of the vanilla bean. *J. Essent. Oil Res.* **1989a**, *1*, 33–41.

Leong, G.; Uzio, R.; Derbesy, M. Synthesis, identification and determination of glucosides present in green vanilla beans (*Vanilla fragrans Andrews*). *Flavour Fragrance J.* **1989b**, *4*, 163–167.

Mane, J.; Zuccha, J. Fr. Pat. Appl. 2 691 880, 1993.

Ranadive, A. S. Vanillin and related flavor compounds in vanilla extracts made from beans of various global origins. *J. Agric. Food Chem.* **1992**, *40*, 1922–1924.

Taylor, S. Improved determination of vanillin and related phenolic components in vanilla (*Vanilla fragrans* (Salisb.) Ames) by high-performance liquid chromatography. *Flavour Fragrance J.* **1993**, *8*, 281–287.

Tokoro, K.; Kawahara, S.; Amano, A.; Kanisawa, T.; Indo, M. In *Flavour Science and Technology*; Bessiere, Y., Thomas, H. F., Eds.; Wiley: New York, 1990; pp 73–75.

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