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Comparative Analysis of Volatiles in Traditionally Cured Bourbon and Ugandan Vanilla Bean (*Vanilla planifolia*) Extracts

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ABSTRACT: Traditionally cured vanilla beans (*Vanilla planifolia*) from Madagascar and Uganda were extracted with organic solvents, and the volatiles were separated from the nonvolatile fraction using the solvent assisted flavor evaporation (SAFE) technique. Concentrated vanilla bean extracts were analyzed using GC-MS and GC-O. Two hundred and forty-six volatile compounds were identified using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software, of which 13 were confirmed with authentic compounds from commercial sources and the others were tentatively identified on the basis of calibrated linear retention indices and the comparison of deconvoluted mass spectra with the in-house and/or NIST spectra databases. Vanillin was the most abundant constituent followed by guaiacol. The total concentration of the volatile compounds, excluding vanillin, was 301 mg/kg for Bourbon and 398 mg/kg for Ugandan vanilla bean extracts. Analytical comparison between the two vanilla bean extracts was discussed. Seventy-eight compounds were identified as odor-active compounds in the vanilla bean extracts with 10 confirmed with authentic references. It was found that there were substantial analytical differences in the odor-active compounds of the two extracts.

KEYWORDS: *traditionally cured vanilla bean extracts, volatiles analysis, solvent-assisted flavor evaporation, GC-MS, GC-O*

INTRODUCTION

Vanilla is one of the most widely used flavors in the world. Most vanilla of international commerce is derived from *Vanilla planifolia* Andrews (synonym *Vanilla fragrans* Ames). Bourbon vanilla, cultivated in Madagascar and other islands in the Indian Ocean, represents approximately 75% of the world production.[1] Uganda is one of the other major vanilla-growing areas.

Vanilla is cultivated from a plant of the orchid family. Its beans or pods are the fruits of the plant, which are harvested when they are fully mature, indicated by pale yellow discoloration at the distal end of the beans. After harvesting, the vanilla beans need to be cured to develop the characteristic flavor. In general, vanilla curing consists of four basic steps: blanching or wilting, sweating/sunning, slow-drying, and conditioning of the beans. The first step, blanching or wilting, is mainly to prevent mold and rot by killing the majority of yeasts and fungi. It is achieved by sun blanching, oven blanching, hot water blanching, or blanching by freezing. In Madagascar, beans are blanched by immersion in hot water (63−65 °C) for 2−3 min. In the next step, sweating, vanilla beans are wrapped in woolen cloth to raise the temperature to 45−65 °C under high humidity and placed in wooden sweating boxes for 24−48 h. These conditions allow enzymes to catalyze the reactions involved in generating the characteristic vanilla color, flavor, and aroma. Then the beans are exposed daily to the sun, dried for 6−8 days to a final 60−70% moisture content by weight. The next step is to slowly dry the beans at room temperature to lower the moisture content to about 25−30% of the total bean weight. This process lasts for about 3−4 weeks. Finally, in the conditioning step, vanilla beans are kept in closed boxes at room temperature for at least 3 months to allow the complete development of aroma. After curing, vanilla beans are sorted and graded in different categories. Then they are bundled and packed into cardboard or tin boxes lined with wax paper, ready for shipment.[1−3]

The elegant vanilla flavor results from a complex mixture of chemical compounds. Vanilla bean volatile compounds have been investigated for decades. In 1976, Klimes and Lamparsky reported that 169 compounds were identified as volatiles in cured Bourbon vanilla beans,[4] of which vanillin is the most abundant. Currently more than 200 volatile compounds are known to occur in cured Bourbon vanilla beans.[5] Liquid extraction is widely used as the extraction method to collect volatiles from the beans.[4,6−9] Hartman et al. have developed direct thermal desorption and applied it to vanilla flavor studies.[10,11] Other sampling techniques include headspace analysis, sorptive stir bar extraction,[6] and solid-phase microextraction (SPME).[12] Gas chromatography (GC) equipped with mass spectrometry (MS) is the most popular analytical method for analysis of the volatiles in vanilla beans after the extracts have been prepared.[6−12] High-performance liquid chromatography (HPLC) has been applied to analyze the major constituents including vanillin, which has lower volatility and usually saturates GC detectors[8,9] when trace compounds are analyzed unless samples are highly diluted.

GC−olfactometry (GC-O) is commonly used in the flavor and fragrance industry.[13−15] Being one of the most popular flavors, vanilla has been widely studied and reviewed.[1,10,16] However, there is very little information published in the literature about odor-active compounds of cured Bourbon and Ugandan vanilla beans. Pérez-Silva et al. reported 26 aroma-active compounds in the pentane/ether extracts of cured vanilla

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Table 1. Volatile Compounds Identified in the Traditionally Cured Bourbon and Ugandan Vanilla Bean Extracts

compound[a]	Bourbon[b] (mg/kg)	Ugandan[b] (mg/kg)	RI_{DB-1} ($RI_{lib\ apol}$)	ID[c]
3-buten-2-one (methyl vinyl ketone)	0.02	0.01	572 (558)	1
2,3-butanedione (diacetyl)	0.17	0.33	573 (566)	1
2-butanone (methyl ethyl ketone)	0.01	0.09	582 (576)	1
hexane	0.13	0.15	589 (600)	1
2-methyl-3-buten-2-ol (dimethyl vinyl carbinol)	0.24	0.18	605 (599)	1
ethyl acetate	0.05	0.04	606 (603)	1
acetic acid	6.04	14.17	613 (611)	1
methyl propionate	N/D[d]	<0.01	620 (612)	1
***tert*-amyl alcohol**	0.02	0.03	631 (625)	1
acetol (hydroxyacetone)	0.08	0.15	632 (630)	1
3-methylbutanal (isovaleraldehyde)	0.25	0.13	637 (627)	1
3-methyl-2-butanone (methyl isopropyl ketone)	3.16	3.29	642 (640)	1
2-methylbutanal	0.12	0.08	647 (645)	1
1-butanol	N/D	0.01	652 (663)	1
cis-3-penten-2-one	0.12	0.08	655 (653)	1
4,5-dihydro-2-methylfuran	0.01	0.01	660 (658)	1
***cis*-3-penten-2-ol**	0.68	0.65	672 (671)	1
valeraldehyde (amyl aldehyde)	0.06	0.01	676 (667)	1
cyclohexene	0.01	0.02	677 (676)	1
propionic acid	0.10	0.44	677 (687)	1
3-hydroxy-2-butanone (acetoin)	5.74	8.57	681 (679)	1
2-ethylfuran	0.01	N/D	692 (691)	1
heptane	<0.01	N/D	702 (700)	1
unidentified	0.01	0.01		1
methyl butyrate	N/D	0.01	708 (708)	1
3-methyl-3-pentanol	0.02	0.01	709 (739)	1
3-pentanol	0.06	0.06	710 (687)	1
trans-3-penten-2-one	0.04	0.01	715 (715)	1
propylene glycol	0.19	0.44	719 (717)	1
isoamyl alcohol	0.18	0.19	722 (725)	1
2-methyl-1-butanol (2-methylbutyl alcohol)	0.01	0.06	726 (725)	1
isobutyric acid	0.02	0.03	743 (738)	1
1-pentanol (amyl alcohol)	0.07	0.05	752 (755)	1
3-methyl-2-butenal (3-methylcrotonaldehyde, senecialdehyde)	N/D	0.02	755 (756)	1
toluene	0.54	0.59	756 (757)	1
3-methyl-2-buten-1-ol (prenol)	0.17	0.10	759 (764)	1
erythro-2,3-butanediol (*anti*-2,3-butanediol, 2,3-butanediol I)	22.13	40.68	762 (759)	1
butanoic acid (butyric acid)	0.21	0.71	772 (774)	1
threo-2,3-butanediol (*syn*-2,3-butanediol, 2,3-butanediol II)	14.38	15.4	772 (767)	1
hexanal	0.57	0.40	778 (778)	1
2-hexanol	0.04	0.09	791 (792)	1
ethyl 2-hydroxyisobutyrate	0.27	0.30	794 (792)	1
unidentified	0.01	N/D		1
octane	0.04	0.35	800 (800)	1
2-furaldehyde (2-furfural)	5.43	10.06	801 (803)	1
4-hexen-3-one	0.02	N/D	813 (811)	1
4-hydroxy-4-methyl-2-pentanone (diacetone alcohol)	0.03	0.03	814 (815)	1
2-furfurol	0.10	N/D	830 (830)	1
isovaleric acid	1.18	1.20	832 (820)	1
***cis*-3-hexen-1-ol (pipol)**	0.05	0.02	839 (841)	1
2-methylbutyric acid	0.61	0.13	841 (843)	1
4-cyclopentene-1,3-dione	0.02	0.02	843 (845)	1
ethylbenzene	0.01	0.01	851 (852)	1
1-hexanol	0.15	0.07	852 (853)	1
2(5*H*)-furanone	0.05	N/D	858 (860)	1
3-methylbutyl acetate (isoamyl acetate)	N/D	0.02	860 (862)	1
γ -butyrolactone	1.46	1.87	859 (861)	1
pentanoic acid (valeric acid)	0.45	0.17	865 (860)	1
3-methyl-2-butenoic acid (3,3-dimethylacrylic acid)	<0.01	0.03	869 (898)	1
2-heptanone (methyl amyl ketone)	0.03	0.01	869 (866)	1

Table 1. continued

compound[a]	Bourbon[b] (mg/kg)	Ugandan[b] (mg/kg)	RI_{DB-1} ($RI_{lib\ apol}$)	ID[c]
styrene	0.01	0.09	877 (878)	1
heptanal	0.03	0.04	879 (877)	1
2-acetylfuran	0.04	0.11	881 (883)	1
2,2,4,4-tetramethyl-3-pentanone	0.03	0.19	883 (883)	1
unidentified	0.02	0.02		1
2-butoxyethanol (butyl cellosolve)	0.02	0.03	888 (889)	1
unidentified	0.02	N/D		1
***erythro*-2,3-butanediol monoacetate (*anti*-2,3-butanediol monoacetate, 2,3-butanediol monoacetate I)**	0.15	0.34	900 (901)	1
dihydro-3-methyl-2(3*H*)-furanone (α-methyl-γ-butyrolactone)	0.07	0.09	901 (904)	1
γ-valerolactone	0.01	0.17	904 (907)	1
methyl caproate (methyl hexanoate)	0.02	0.02	905 (906)	1
***threo*-2,3-butanediol monoacetate (*syn*-2,3-butanediol monoacetate, 2,3-butanediol monoacetate II)**	0.31	0.39	906 (901)	1
3-methylvaleric acid	N/D	0.14	925 (925)	1
5-methyl-2-furfural	0.41	1.39	930 (934)	1
benzaldehyde	0.35	0.49	932 (937)	1
α-pinene	0.02	0.01	936 (938)	1
isopropylbenzene (cumene)	0.01	<0.01	951 (962)	1
1-heptanol	0.09	0.09	953 (956)	1
phenol	9.71	9.97	956 (957)	1
hexanoic acid (caproic acid)	0.05	0.38	959 (957)	1
1-octen-3-ol	0.83	0.13	964 (966)	1
2-octanone (methyl hexyl ketone)	0.11	N/D	970 (971)	1
unidentified	0.23	0.34		1
2-pentylfuran	0.32	N/D	980 (984)	1
octanal	0.11	N/D	981 (979)	1
1,2,4-trimethylbenzene (pseudocumene)	0.04	0.01	984 (980)	1
3-ethoxyhexanal	0.07	N/D	989 (989)	1
5-ethyl-2(5*H*)-furanone (2-hexen-4-olide)	0.07	N/D	991 (961)	1
3,4-dimethyl-2,5-furandione	N/D	0.07	993 (987)	1
1,1′-dipropylene glycol 2′-methyl ether	N/D	0.01	996 (992)	1
2-hydroxy-3,3-dimethyl-γ-butyrolactone (pantolactone)	5.15	6.25	999 (998)	1
unidentified	0.02	N/D		1, 2
benzyl alcohol	4.30	3.38	1008 (1008)	1
γ-hexalactone	0.03	0.08	1011 (1011)	1
phenylacetaldehyde	0.04	N/D	1012 (1015)	1
3-octen-2-one	0.37	0.09	1015 (1016)	1
p-isopropyltoluene (*p*-cymene)	0.01	0.03	1016 (1021)	1
2-hydroxybenzaldehyde (salicylaldehyde)	0.06	0.09	1016 (1020)	1
2,2,6-trimethylcyclohexanone	0.02	0.01	1019 (1021)	1
limonene	0.11	0.12	1026 (1028)	1
unidentified	0.17	0.08		1
2-methylphenol (*o*-cresol)	0.01	0.08	1030 (1028)	1
2-furoic acid (2-furancarboxylic acid)	0.02	0.19	1034 (1036)	1
acetophenone	0.02	0.06	1039 (1042)	1
3,5-octadien-2-one	0.11	0.02	1044 (1046)	1
4-methylphenol (*p*-cresol)	3.74	1.69	1050 (1047)	1
2-(hydroxyacetyl)furan	0.07	0.11	1053 (1053)	1
2-octen-1-ol	0.20	N/D	1054 (1052)	1
heptanoic acid (oenanthic acid)	0.35	0.30	1054 (1061)	1
1-octanol	1.00	0.31	1056 (1053)	1
guaiacol	105.0	169.5	1069 (1070)	1
unidentified	0.04	0.05		1, 2
methyl benzoate	N/D	0.03	1075 (1068)	1
6-methyl-3,5-heptadien-2-one	0.06	0.04	1079 (1081)	1
3-hydroxy-2-methylpyran-4-one (maltol, corps praline)	0.51	0.58	1080 (1084)	1
unidentified	0.02	N/D		1
nonanal	0.26	0.32	1084 (1085)	1
phenethanol	1.55	0.84	1089 (1095)	1
2-ethylhexanoic acid	N/D	0.02	1096 (1103)	1

Table 1. continued

compound[a]	Bourbon[b] (mg/kg)	Ugandan[b] (mg/kg)	RI_{DB-1} ($RI_{lib\ apol}$)	ID[c]
undecane	0.03	0.04	1099 (1100)	1
methyl octanoate (methyl caprylate)	0.08	0.07	1105 (1100)	1
2-vinylanisole (2-methoxystyrene)	0.01	0.01	1109 (1115)	1
unidentified	N/D	0.20		1
1,2-dimethoxybenzene (veratrole)	0.46	0.14	1112 (1113)	1
4-methyl-5,6-dihydro-2-pyranone (dehydromevalonolactone)	0.06	0.41	1113 (1115)	1
unidentified	<0.01	N/D		1
2,4-dimethylphenol (2,4-xylenol)	0.01	0.01	1124 (1130)	1
benzyl acetate	0.03	0.09	1135 (1136)	1
benzoic acid	0.64	1.57	1138 (1129)	1
octanoic acid (caprylic acid)	1.53	0.62	1150 (1157)	1
4-ethylbenzaldehyde	N/D	0.01	1154 (1148)	1
1-nonanol	0.10	0.04	1155 (1155)	1
3,5-dihydroxy-2-methylpyran-4-one (hydroxymaltol)	0.21	0.38	1161 (1160)	1
2-methoxy-4-methylphenol (4-methylguaiacol, creosol)	5.55	7.71	1170 (1173)	1
naphthalene	0.05	0.05	1173 (1177)	1
methyl salicylate	1.75	0.51	1176 (1179)	1
α-terpineol	0.02	N/D	1180 (1181)	1
5-(hydroxymethyl)-2-furfural	0.40	0.47	1183 (1181)	1, 2
dehydro-β-cyclocitral (safranal)	0.09	0.07	1183 (1186)	1
p-vinylphenol	0.04	0.02	1185 (1185)	1
4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one (verbenone, 2-pinen-4-one)	0.12	N/D	1191 (1187)	1
octyl acetate	N/D	0.05	1191 (1192)	1
unidentified	0.60	0.11		1
dodecane	0.03	0.20	1199 (1200)	1
unidentified	<0.01	0.05		1
3-phenylfuran	0.01	0.33	1203 (1208)	1
methyl nonanoate (methyl pelargonate)	0.14	0.46	1205 (1208)	1
3-phenyl-1-propanol	0.30	0.48	1206 (1201)	1
1,2-dimethoxy-4-methylbenzene (methyl creosol)	0.07	0.07	1206 (1212)	1
phenylacetic acid	0.07	N/D	1214 (1220)	1
γ-octalactone	0.07	0.03	1218 (1221)	1
4-methoxybenzaldehyde (*p*-anisaldehyde)	0.41	6.09	1221 (1220)	1, 2
4-allylphenol (chavicol)	0.16	0.04	1223 (1224)	1
phenethyl acetate	0.02	0.02	1229 (1233)	1
trans-cinnamaldehyde	0.13	0.07	1239 (1243)	1
nonanoic acid (pelargonic acid)	2.94	2.52	1247 (1244)	1, 2
methyl 3-phenylpropionate	N/D	0.12	1250 (1255)	1
p-methoxybenzyl alcohol (anisyl alcohol)	4.43	0.92	1253 (1249)	1
4-ethylguaiacol	0.04	N/D	1257 (1262)	1
p-hydroxybenzyl methyl ether (α-methoxy-*p*-cresol)	0.49	N/D	1266 (1272)	1
***o*-vanillin (3-methoxysalicylaldehyde)**	0.01	N/D	1273 (1276)	1
methyl *cis*-cinnamate	0.53	4.10	1276 (1278)	1
cinnamyl alcohol	0.80	3.04	1278 (1281)	1
3-methyl-5-propyl-2-cyclohexen-1-one (celery ketone, livescone)	0.27	N/D	1284 (1295)	1,3
1,4-benzenediol (hydroquinone)	0.08	N/D	1284 (1298)	1
1-methylnaphthalene	0.02	0.03	1286 (1290)	1
2-methoxy-4-vinylphenol	0.06	0.09	1288 (1294)	1
***cis*-dihydroedulan (dihydroedulan II)**	0.01	<0.01	1293 (1298)	1
tridecane	0.05	0.09	1300 (1300)	1
heliotropine (piperonal)	0.05	0.05	1301 (1308)	1, 2
2-methylnaphthalene	0.01	0.01	1302 (1306)	1
methyl decanoate (methyl caprate)	0.02	0.04	1309 (1308)	1, 2
2,6-dimethoxyphenol (pyrogallol 1,3-dimethyl ether)	0.03	N/D	1315 (1325)	1
γ-nonalactone	0.34	N/D	1325 (1324)	1
benzylidene acetone (4-phenyl-3-buten-2-one)	<0.01	N/D	1328 (1333)	1
4-allyl-2-methoxyphenol (eugenol, 4-allylguaiacol)	0.11	N/D	1334 (1338)	1, 2
p-hydroxybenzaldehyde	27.06	8.61	1335 (1315)	1, 2
decanoic acid (capric acid)	0.10	N/D	1346 (1344)	1
methyl *p*-methoxybenzoate (methyl *p*-anisate)	0.44	N/D	1347 (1350)	1

Table 1. continued

compound[a]	Bourbon[b] (mg/kg)	Ugandan[b] (mg/kg)	RI_{DB-1} ($RI_{lib\ apol}$)	ID[c]
methyl *trans*-cinnamate	6.56	23.28	1360 (1361)	1
vanillin	sat.	sat.	1368 (1360)	1
vanillyl methyl ether	0.15	N/D	1377 (1374)	1
α-copaene	0.11	0.15	1392 (1392)	1
tetradecane	N/D	0.04	1406 (1400)	1
2,5-dihydroxybenzaldehyde	0.12	N/D	1407 (1420)	1
***iso*-vanillin**	1.13	0.07	1413 (1426)	1, 2
trans-cinnamic acid	0.17	1.65	1413 (1387)	1
***cis*-α-bergamotene**	0.10	N/D	1425 (1422)	1
α-gurjunene	N/D	0.12	1427 (1424)	1
methyl 4-hydroxybenzoate (methylparaben)	0.38	1.56	1429 (1425)	1
2-ethylnaphthalene	0.05	0.12	1430 (1444)	1
α-santalene	0.06	N/D	1433 (1431)	1
4-hydroxy-3-methoxybenzyl alcohol (vanillyl alcohol)	0.61	0.08	1437 (1412)	1
β-caryophyllene	N/D	0.19	1442 (1440)	1
vanillyl alcohol ethyl ether (vanillyl ethyl ether)	0.09	N/D	1443 (1441)	1
***trans*-α-bergamotene**	0.23	N/D	1445 (1446)	1
ethyl *trans*-cinnamate	0.04	1.09	1447 (1445)	1
acetovanillone (apocynin)	1.49	0.82	1469 (1458)	1, 4
α-caryophyllene (α-humulene)	N/D	0.02	1472 (1472)	1
α-D-curcumene	0.08	0.02	1480 (1480)	1
germacrene D	0.10	0.12	1486 (1496)	1
vanillin acetate	0.46	0.21	1495 (1484)	1
methyl vanillate	0.42	1.18	1496 (1482)	1
pentadecane	0.06	0.02	1500 (1500)	1
3,4-dimethyl-5-pentylidene-2(5*H*)-furanone (bovolide)	0.04	N/D	1504 (1506)	1
4-hydroxy-3-methoxyphenylacetone (methyl vanillyl ketone, guaiacylacetone)	0.04	0.06	1507 (1498)	1
γ-cadinene	0.13	0.07	1507 (1510)	1
methyl dodecanoate (methyl laurate)	0.08	0.08	1508 (1508)	1
valencene	0.27	0.03	1510 (1510)	1
calamenene	0.02	0.03	1526 (1528)	1
δ-cadinene	0.07	0.10	1528 (1531)	1
4-hydroxy-3-methoxybenzoic acid (vanillic acid)	0.57	0.48	1530 (1518)	1
α-calacorene	0.03	0.04	1546 (1550)	1
4-ethoxy-3-methoxybenzaldehyde (vanillin ethyl ether, 4-ethoxy-3-anialdehyde)	0.17	0.26	1559 (1548)	1
diethyl phthalate	0.07	N/D	1561 (1562)	1
trans-nerolidol (tentative)	0.02	0.29	1571 (1554)	1
hexadecane	0.09	0.08	1599 (1600)	1
3,5-dimethoxy-4-hydroxybenzaldehyde (syringaldehyde, 5-methoxyvanillin)	0.17	0.35	1618 (1609)	1, 2
***erythro*-vanillin-propylene glycol acetal (*anti*-vanillin-propylene glycol acetal, vanillin-propylene glycol acetal I)**	0.18	0.18	1652 (1646)	1
***threo*-vanillin-propylene glycol acetal (*syn*-vanillin-propylene glycol acetal, vanillin-propylene glycol acetal II)**	0.12	0.15	1657 (1651)	1
erythro-vanillin 2,3-butanediol acetal (*anti*-vanillin 2,3-butanediol acetal, vanillin 2,3-butanediol acetal I)	11.83	6.92	1679 (1683)	1
heptadecane	0.07	0.04	1699 (1700)	1
threo-vanillin 2,3-butanediol acetal (*syn*-vanillin 2,3-butanediol acetal, vanillin 2,3-butanediol acetal II)	17.23	7.20	1719 (1683)	1
octadecane	0.06	0.04	1799 (1800)	1
6,10,14-trimethyl-2-pentadecanone	0.23	0.04	1832 (1832)	1
nonadecane	0.07	0.02	1899 (1900)	1
methyl hexadecanoate (methyl palmitate)	0.06	0.26	1907 (1909)	1
dibutyl phthalate	0.07	0.13	1926 (1932)	1
ethyl palmitate	0.01	0.02	1976 (1977)	1
methyl *trans*-9,*trans*-12-octadecadienoate (methyl linolelaidate)	N/D	0.14	2074 (2075)	1
cembrene	0.02	0.28	2079 (2072)	1
heneicosane	0.06	0.04	2099 (2100)	1
***p*-(*p*-hydroxyphenoxy)benzoic acid**	0.17	0.03	2123 (2133)	1
docosane	0.05	0.04	2199 (2200)	1
cis-9-tricosene	0.03	0.07	2273 (2276)	1
tricosane	0.17	0.22	2299 (2300)	1
hexanedioic acid, bis(2-ethylhexyl) ester	0.55	0.06	2373 (2382)	1

Table 1. continued

compound[a]	Bourbon[b] (mg/kg)	Ugandan[b] (mg/kg)	RI_{DB-1} ($RI_{lib\ apol}$)	ID[c]
tetracosane	0.04	0.06	2399 (2400)	1
pentacosane	0.06	0.12	2499 (2500)	1
dioctyl phthalate (bis(2-ethylhexyl) phthalate)	0.08	0.22	2515 (2521)	1
***cis*-18-heptacosene-2,4-dione**	2.26	7.26	2978 (2988)	1
***cis*-20-nonacosene-2,4-dione**	0.32	1.39	3234 (−)	1
total	300.8	397.8		

[a]Compounds in bold are not previously reported as constituents in vanilla bean extracts. [b]mg substance per kg wet vanilla beans. [c](1) Tentative identification was based on RI (calibrated with C5−C30 alkanes) and EI mass spectral comparison with in-house and/or NIST libraries. (2) Identification was based on RI and EI mass spectral comparison with standards from Sigma-Aldrich. (3) Identification was based on RI and EI mass spectral comparison with standards from Firmenich. (4) Identification was based on RI and EI mass spectral comparison with standards from SAFC. [d]N/D, not detected.

(*V. planifolia* G. Jackson) beans.[7] Odor-active compounds are volatile compounds that could be perceived, either pleasantly or unpleasantly, by human beings at certain concentrations. Aroma refers to a pleasant odor, especially in the flavor and fragrance industry.

The objective of this study was to use liquid extraction and distillation techniques to extract and separate the volatile compounds from the vanilla beans of Bourbon and Ugandan origins and to analyze the differences in the total composition and odor-active compounds of the two extracts using GC-MS and GC-O to gain knowledge on the contributing factors affecting the aromatic quality of traditionally cured vanilla beans from different origins.

MATERIALS AND METHODS

Materials. Traditionally cured vanilla beans, all 2007 harvested crops, were provided by Krishna Balasundaram at Firmenich, St. Louis, MO, USA. Ugandan vanilla beans (Kampala) are harvested in two seasons per year, but the beans used in this study were from the June/July harvest. Bourbon vanilla beans (Antalaha) were harvested in the June−August season of one year. The precut beans mentioned here were cut in St. Louis, MO, before shipment to Princeton, NJ. The beans were stored at −80 °C before and after experiments.

The following organic solvents were of 99+% purity: dichloromethane (DCM) stabilized with amylene (Burdick and Jackson, distributed by VWR International, USA); anhydrous pentane and anhydrous diethyl ether with 1 mg/kg BHT as inhibitor (Sigma-Aldrich, Milwaukee, WI, USA).

Thirteen standard compounds from commercially available sources, which are listed in Table 1, were at least 98% pure, except for nonanoic acid, which was at least 96% pure.

Inorganic chemicals, including 36.5−38.0% hydrochloric acid (J. T. Baker), ACS grade sodium hydroxide (J. T. Baker), and anhydrous ACS grade sodium sulfate (Mallinckrodt Chemicals), were purchased from VWR International, USA. Anhydrous sodium carbonate (≥99.5%, ACS reagent, granular) was obtained from Sigma-Aldrich (Milwaukee, WI, USA).

Measurement of Water Content. A sample of precut vanilla beans (5.0 g) was sliced into pieces (<0.5 cm long) and heated on an aluminum pan in a Mettler Toledo infrared dryer LP16-m (Mettler-Toledo, Greifensee, Switzerland) at 100 °C for 4 h until constant weight reached. The water content of the sample was calculated on the basis of the weight loss of the original or wet sample. Duplicates of each sample were measured.

Extraction. Precut beans or whole beans (cut into pieces <1 in. long) were frozen in liquid N_2 in a stainless steel beaker and then ground with a KitchenAid BCG100 blade coffee grinder to a fine powder. A portion of vanilla bean powder (10 g) was charged into a 50 mL glass centrifuge tube, followed by the addition of 5.0 mL of deionized water and 40.0 mL of DCM. The mixture was shaken horizontally at the maximum speed on a Genie2 vortex mixer (Scientific Industries, Inc., Bohemia, NY, USA) for 30 min. The mixture was filtered over a tablespoon of Na_2SO_4 in a glass funnel lined with a folded filter paper (Whatman, Sharkskin, distributed by VWR International, USA). The bean powder was rinsed with 10 mL of DCM three times. The combined brown DCM extract was blanketed under N_2 and stored at −20 °C before solvent-assisted flavor evaporation (SAFE) distillation.

SAFE. The SAFE apparatus was the same as described in Engel's paper.[18] The above brown extract was charged into the hopper and slowly introduced into a 1 L flask by opening the valve. The 1 L flask was heated by a water bath at a temperature of 41 °C (set at 45 °C). The distillation was performed under vacuum ($(4-5) \times 10^{-4}$ mbar).

Concentration of the SAFE Distillate. After the SAFE distillate had warmed up to room temperature, about 30−40 mL of DCM was removed using a rotary evaporator at 50 °C under atmospheric pressure. The remaining solution was transferred to a 200 mL sample tube and concentrated to 0.5 mL under a N_2 flow, using a TurboVap concentrator (Caliper Life Sciences, Hopkinton, MA, USA). The water bath temperature was 38 °C, and the N_2 pressure was 1.4−1.6 Pa.

GC-MS and GC-O. An Agilent GC 6890N equipped with an MSD-5975 was used for GC-MS and GC-O analysis. Inside the GC, a column was connected to a cross splitter (purchased from VICI Valco Instruments Co. Inc., Houston, TX, USA), which split the effluent coming out of the column into three detectors, namely, MSD, FPD, and GC-O sniffing port. The transfer line to the sniffing port was heated to 300 °C to prevent condensation. A nonpolar fused silica capillary column (Restek Rxi-1, 0.32 mm i.d. × 60 m length × 1.0 μm film thickness) was used with the following temperature program: 40 °C was set as the initial temperature and maintained for 5 min; it was raised to 300 °C at a rate of 4 °C/min and held at 300 °C for 20 min. A constant flow rate of the carrier gas (He), at 3.8 mL/min, was applied in this method. A polar fused silica capillary column (Restek Stabilwax, 0.32 mm i.d. × 30 m length × 1.0 μm film thickness) was also used with the following temperature program: 40 °C was set as the initial temperature and maintained for 5 min; then it was raised to 240 °C at a rate of 4 °C/min and held at 240 °C for 20 min. A constant flow rate of the carrier gas (He), at 4.9 mL/min, was applied in this method. A volume of 2.0 μL of each sample was injected splitlessly. The electron impact energy was 70 eV. Electron ionization (EI) mass spectra were recorded in the range m/z 35−425 at 3 spectra/s. Temperatures for the MS source and quadrapole were 230 and 150 °C, respectively. Compound identification was based on linear retention index (RI), which was calculated using *n*-alkanes (C5−C30) as reference compounds, and comparison of mass spectra with the in-house and/or NIST spectra databases. Thirteen commercially available standards were injected into GC-MS using the same conditions to confirm the identification. No correction for individual response factors was performed. Two or three panelists took turns sniffing during the GC-O runs for 20−30 min. The panelists' descriptions of the aromas were mostly compared with the available descriptors in the

Flavor Raw Materials database (Boelens Aroma Chemical Information Service, The Netherlands). Each sample was analyzed in triplicate.

RESULTS AND DISCUSSION

Extraction and SAFE. To retain the similarity of the extracts to the original vanilla beans, it is important to choose low-boiling solvents for extraction, which can easily be removed without loss of very volatile flavor compounds. Klimes and Lamparsky found that absolute methanol was an appropriate solvent for extracting aroma compounds in vanilla beans. In their case, most methanol was removed, and then the concentrate was subsequently treated with peroxide-free diethyl ether, followed either by repeated freezing at −25 °C and decantation or by Kugelrohr distillation at a temperature of 160 °C under 0.05 Torr pressure to separate the volatiles from the nonvolatiles. According to Klimes and Lamparsky, distillation at 160 °C was not detrimental to the sensorial quality of the distillate.[4] Peréz-Silva et al. compared diethyl ether, pentane/diethyl ether (P/E) (1:1, v/v), and pentane/dichloromethane (2:1, v/v) for extraction of vanilla volatiles.[7] Their study showed that P/E gave the highest number (65) of volatile compounds. They also stated that adding a small, controlled amount of water favored the extraction of volatiles. They reported two layers after extraction with the addition of water. It appeared that they discarded the aqueous layer without further analysis.

At the beginning of this study, traditionally cured whole Bourbon vanilla beans were used for development of the extraction method. The ground vanilla bean powders were extracted with pentane/diethyl ether (1:1, v/v) or dichloromethane with the addition of some water (8:1, v/v). Different from Peréz-Silva's paper,[7] the extracts contained only one layer after the beans were filtered. Most of the water had been absorbed by the beans. DCM was chosen for the extractions of cured vanilla beans in this study due to our interest in more polar compounds and a slightly stronger olfactive impact from the DCM extract. After extraction and SAFE distillation, there was a dark brown, viscous, resin-like residue left in the distillation pot, which was ca. 10% w/w of wet weight of the vanilla beans before extraction.

Comparative Analysis of the Volatile Compounds in the Traditionally Cured Bourbon and Ugandan Vanilla Bean Extracts. After the extraction and separation methods had been developed, precut traditionally cured Bourbon and Ugandan vanilla beans were first analyzed for their moisture contents. The average water contents were 18.3 and 20.7% w/w for the precut traditionally cured Bourbon and Uganda vanilla beans, respectively. It seemed that there was no significant difference in water content between the batches of these two analyzed vanilla beans. However, this result was based on only one batch sample for each of the beans as described under Materials and Methods.

Then the vanilla bean extracts were analyzed using GC-MS and GC-O. Two hundred and forty-six compounds were found in the two vanilla bean extracts. They belonged to the following chemical classes: hydrocarbons, aldehydes, ketones, alcohols, phenols, acids, esters/lactones, ethers, and heterocyclics. Chlorocyclohexane (10 mg/kg wet weight of the beans) was added to the beans during extraction as an internal standard (IS) and subjected to the SAFE and concentration steps along with the other volatiles. Component concentrations were calculated on the basis of the ratio of the total deconvoluted area of each component against that of the internal standard, using the Automated Mass Spectral Deconvolution and Identification System (AMDIS). In more detail, AMDIS deconvolutes coeluting compounds by using some characteristic ions as model ions and gathering coapexed or comaximized ions for its mass spectrum. Then the software sums the ion intensity or ion counts of all the coapexed ions as the total deconvoluted area of that compound. The mass ratio or concentration (mg/kg) of each volatile compound was based on the wet weight of the vanilla beans. All volatile compounds identified using an apolar column are summarized in Table 1. There is only one set of measured RI values listed in Table 1 because the measured RI values were the same for both vanilla bean extracts, taking into consideration the instrumental variation. Among the 246 compounds in Table 1, 13 were confirmed with authentic compounds from commercial sources, and the others were tentatively identified on the basis of calibrated linear retention indices and the comparison of deconvoluted mass spectra with the in-house and/or NIST spectra databases. There were 109 compounds in Table 1 as first time identified constituents in vanilla bean extracts (5 of them were confirmed with authentic compounds).

The concentration of vanillin was so high that it saturated the MS detector. However, the vanillin concentration, 1.5−2.5% in Bourbon vanilla beans[8−10] and 1.5−2.0% in Ugandan vanilla beans,[8] has been very well studied and published in the literature. We did not want to repeat the measurement of vanillin in this study but rather concentrated on the other volatile compounds.

Next to vanillin, guaiacol was the second most abundant volatile compound in both Bourbon and Ugandan vanilla bean extracts, with mass ratios of 105 and 170 mg/kg, respectively. Pérez-Silva et al. reported 9.3 ppm guaiacol in the vanilla bean (*V. planifolia* G. Jackson) from the Tuxtepec region of Mexico using P:E (1:1, v/v) and water as the extracting solvents.[7] Using the DTD-GC method, no guaiacol was reported in six vanilla samples from different origins including Madagascar, Bali, Tahiti, and Java,[11] whereas 19 ppm guaiacol was reported in the cured Bourbon vanilla beans.[10] Differences of guaicol concentration within the literature could be caused by the beans from different regions and/or different methods for sample preparation.

Compounds with mass ratios between 10 and 100 mg/kg in either one of the two beans included acetic acid, 2,3-butanediol *erythro (anti)*- and *threo (syn)*- diastereomers, 2-furaldehyde, *p*-hydroxybenzaldehyde, methyl *trans*-cinnamate, and vanillin 2,3-butanediol acetal *erythro (anti)*- and *threo (syn)*- diastereomers. The mass ratios of the following compounds were in the range of 1−10 mg/kg in either one of the two beans: 3-methyl-2-butanone, acetoin, isovaleric acid, γ-butyrolactone, phenol, pantolactone, benzyl alcohol, 4-methylphenol, 1-octanol, phenethanol, benzoic acid, octanoic acid, 4-methylguaiacol, methyl salicylate, *p*-anisaldehyde, nonanoic acid, anisyl alcohol, cinnamyl alcohol, isovanillin, *trans*-cinnamic acid, ethyl *trans*-cinnamate, acetovanillone, methyl vanillate, *cis*-18-heptacosene-2,4-dione, and *cis*-20-nonacosene-2,4-dione. The other compounds were found at <1 mg/kg in these two bean extracts.

p-Hydroxybenzoic acid is one of the most abundant compounds in the vanilla beans and is often measured to monitor the ratio versus vanillin for determination of the authenticity of vanilla beans. However, a recent study carried out by scientists at Givaudan indicated that the so-called "ratios" in the current form are not suitable authenticity parameters.[8] *p*-Hydroxybenzoic acid was not detected in this

Table 2. Odor-Active Compounds in Traditionally Cured Bourbon and Uganda Vanilla Bean Extracts

				Bourbon tradit			Ugandan tradit		
compound[a−c]	descriptor[d]	RI_{DB-1}[e] ($RI_{lib\ apol}$)	RI_{wax}[e] ($RI_{lib\ pol}$)	concn[f] (mg/kg)	odor strength[g] (DB-1)	odor strength[g] (WAX)	concn[f] (mg/kg)	odor strength[g] (DB-1)	odor strength[g] (WAX)
2,3-butanedione (diacetyl)	sweet, buttery, creamy, milky	573 (566)	1005 (988)	0.17	n/d	n/d	0.33	w	m
acetic acid	sour, vinegar-like	613 (611)	1477 (1496)	6.04	n/d	s	14.17	m	s
acetol (hydroxyacetone)	aromatic, caramellic	632 (630)	1326 (1283)	0.08	n/d	m	0.15	n/d	m
3-methylbutanal (isovaleraldehyde)	acrid, fruity, peach- and cocoa-like	637 (627)	N/A (910)	0.25	w	n/d	0.13	w	n/d
isoamyl alcohol	fresh, ethereal, fusel-like, fermented and yeasty	722 (725)	1210 (1193)	0.18	w	n/d	0.19	w	n/d
2,3-butanediol I	soft ethereal	762 (759)	1562 (1529)	22.13	n/d	s	40.68	n/d	s
2,3-butanediol II	soft ethereal	772 (767)	1597 (1563)	14.38	n/d	s	15.40	n/d	s
butanoic acid (butyric acid)	penetrating, reminiscent of rancid butter	772 (774)	1658 (1618)	0.21	n/d	m	0.71	n/d	s
hexanal	green, fruity, aldehydic; somewhat green apple-like	778 (778)	1105 (1083)	0.57	w	m	0.40	w	s
2-furaldehyde (2-furfural)	sweet caramel-like, nutty, baked bread, almond	801 (803)	1491 (1467)	5.43	w	n/d	10.06	w	s
2-furfurol[a]	burnt, sweet, caramellic, brown	830 (830)	1685 (1663)	0.10	w	n/d	N/D	n/d	n/d
isovaleric acid	acidic, cheese-like	832 (820)	1697 (1672)	1.18	s	s	1.20	s	s
2-methylbutyric acid	acidic, sweaty	841 (843)	1698 (1663)	0.61	w	s	0.13	n/d	m
1-hexanol	roasty, nutty; pleasant cheesy	852 (853)	1369 (1355)	0.15	n/d	n/d	0.07	w	n/d
pentanoic acid (valeric acid)	strongly acidic, caprylic	865 (860)	1767 (1737)	0.45	n/d	m	0.17	n/d	n/d
2-hydroxyisobutyric acid	suffocating odor	N/A (867)	1535 (N/A)	0.43	n/d	s	N/D	n/d	n/d
2-acetylfuran	balsamic	881 (883)	1532 (1513)	0.04	n/d	n/d	0.11	n/d	s
benzaldehyde	sweet aromatic, spicy; bitter almond- and dark cherry-like	932 (937)	N/A (1511)	0.35	n/d	n/d	0.49	w	n/d
phenol	strongly phenolic, medicinal	956 (957)	2038 (1982)	9.71	s	m	9.97	s	m
5-ethyl-2(5*H*)-furanone (2-hexen-4-olide)[a]	rice, fruity	991 (961)	1795 (1760)	0.07	n/d	m	N/D	n/d	n/d
2-hydroxy-3,3-dimethyl-γ-butyrolactone (pantolactone)	burnt sugar	999 (998)	2067 (2035)	5.15	n/d	n/d	6.25	m	n/d
unidentified	roasted, somewhat burnt, hazel nut-like			0.02	n/d	m	N/D	n/d	w
benzyl alcohol	chemical, fruity with balsamic nuances	1008 (1008)	1904 (1862)	4.31	n/d	n/d	3.38	n/d	m
2-hydroxybenzaldehyde (salicylaldehyde)	spicy, medicinal and astringent	1016 (1020)	1714 (1685)	0.06	n/d	m	0.09	n/d	w
unidentified	sweet musty, nutty and tea-like			0.17	n/d	n/d	0.08	m	n/d
acetophenone	sweet aromatic, almond-like, nutty, benzaldehyde, with musty fruity nuances	1039 (1042)	1682 (1650)	0.02	n/d	n/d	0.06	w	n/d
3,5-octadien-2-one	fruity green grassy	1044 (1046)	1547 (1570)	0.11	n/d	s	0.02	n/d	s
4-methylphenol (*p*-cresol)	phenolic	1050 (1047)	2114 (2097)	3.74	m	n/d	1.69	w	n/d
heptanoic acid (oenanthic acid)	sour, fatty	1054 (1061)	1979 (1956)	0.35	s	n/d	0.30	w	n/d
1-octanol	roasty	1056 (1053)	1576 (1552)	1.00	w	n/d	0.32	w	n/d
guaiacol (2-methoxyphenol)	aromatic, phenolic, burnt	1066 (1069)	1893 (1865)	105.0	s	s	169.5	s	s
unidentified	roasted aroma, nutty			0.04	w	n/d	0.05	s	n/d
maltol (corps praline)	sweet aromatic, caramellic	1080 (1084)	1995 (1965)	0.51	s	m	0.58	w	n/d
nonanal	aldehydic, peely, floral (somewhat rosy)	1084 (1085)	1417 (1399)	0.26	n/d	m	0.32	n/d	n/d
phenethanol	honey, fruity, sweet floral-rose	1089 (1095)	1941 (1915)	1.55	s	m	0.84	m	m
methyl octanoate (methyl caprylate)	fruity, fatty	1105 (1100)	N/A (1385)	0.08	w	n/d	0.07	n/d	n/d

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Table 2. continued

compound[a−c]	descriptor[d]	RI_{DB-1}[e] ($RI_{lib\ apol}$)	RI_{wax}[e] ($RI_{lib\ pol}$)	Bourbon tradit concn[f] (mg/kg)	Bourbon tradit odor strength[g] (DB-1)	Bourbon tradit odor strength[g] (WAX)	Ugandan tradit concn[f] (mg/kg)	Ugandan tradit odor strength[g] (DB-1)	Ugandan tradit odor strength[g] (WAX)
1,2-dimethoxybenzene (veratrole)	aromatic, somewhat phenolic, medicinal; slightly spicy	1112 (1113)	1753 (1725)	0.46	w	m	0.14	n/d	n/d
caprylic acid (octanoic acid)	caprylic, fatty, oily	1150 (1157)	2086 (2057)	1.53	m	n/d	0.62	w	n/d
1-nonanol	orange, floral, oily, citronella-like	1155 (1155)	1678 (1657)	0.10	m	m	0.04	m	n/d
hydroxymaltol	honey, toasty caramel	1161 (1160)	N/A (1442)	0.21	w	n/d	0.38	m	n/d
2-methoxy-4-methylphenol (4-methylguaiacol, creosol)	powerful cresylic	1170 (1173)	1988 (1953)	5.55	w	m	7.71	w	m
methyl salicylate	medicinal, phenolic, sweet, characteristic wintergreen	1176 (1179)	1811 (1780)	1.75	w	s	0.51	n/d	n/d
unidentified	sweet; nutty			0.60	w	m	0.11	w	n/d
1,2-dimethoxy-4-methylbenzene (methyl creosol)	candy sweet	1206 (1212)	1835 (1807)	0.07	n/d	m	0.07	n/d	n/d
methyl nonanoate (methyl pelargonate)	oily, fatty; slightly fruity	1205 (1208)	1512 (1495)	0.14	w	n/d	0.46	m	m
γ-octalactone	sweet creamy with coconut character	1218 (1221)	1954 (1924)	0.07	s	m	0.03	m	m
4-methoxybenzaldehyde (*p*-anisaldehyde)	sweet, herbaceous-spicy; creamy, powdery, vanilla with a typical marsh-	1221 (1220)	2064 (2020)	0.41	w	m	6.09	s	m
4-(2-propenyl)-phenol (chavicol, 4-allylphenol)	aromatic spicy, somewhat medicinal, phenolic	1223 (1224)	2370 (2329)	0.16	s	n/d	0.04	m	n/d
phenethyl acetate	sweet, floral, fruity, green, rose, dried fruit	1229 (1233)	1845 (1818)	0.02	w	n/d	0.02	w	n/d
trans-cinnamaldehyde	sweet aromatic spicy, cinnamic and cassia-like, balsamic	1239 (1243)	2079 (2040)	0.13	m	m	0.07	m	n/d
nonanoicacid (pelargonic acid)	oily, fatty, caprylic, cheesy	1247 (1244)	2191 (2172)	2.94	w	n/d	2.52	n/d	w
4-methoxybenzylalcohol (anisyl alcohol)	sweet aromatic, balsamic, caramel, nutty	1253 (1249)	2313 (2280)	4.43	w	m	0.92	m	m
methyl *cis*-cinnamate	fruity, balsamic, somewhat strawberry-like	1276 (1278)	1986 (1962)	0.53	m	n/d	4.10	n/d	m
3-phenol-2-propen-1-ol (cinnamyl alcohol)	sweet-warm balsamic, slightly cinnamon	1278 (1281)	2203 (2288)	0.80	m	m	3.04	n/d	n/d
5-isopropyl-2-methyl phenol (carvacrol)[b,c]	spicy, somewhat herbal phenolic	N/A (1284)	2229 (2221)	N/D	n/d	n/d	0.12	n/d	m
3-methyl-5-propyl-2-cyclohexen-1-one (celery ketone, livescone)[a]	slightly sweet, warm, celery like	1284 (1295)	N/A (1830)	0.27	w	n/d	N/D	n/d	n/d
2-methoxy-4-vinylphenol (varamol 106)	aromatic, spicy, somewhat phenolic	1288 (1294)	2232 (2184)	0.06	m	s	0.09	n/d	n/d
dihydroedulan II	sweet, rose like	1293 (1298)	N/A (1538)	0.01	w	n/d	<0.01	n/d	n/d
heliotropine (piperonal)	cherry, powdery, vanilla and sweet anisic	1301 (1308)	2273 (2233)	0.05	n/d	m	0.05	n/d	n/d
methyl decanoate (methyl caprate)	winey, slightly sweet, honey like	1309 (1308)	1614 (1579)	0.02	s	n/d	0.04	s	m
γ-nonalactone[a]	creamy-fatty, coconut-and apricot-like	1325 (1324)	2068 (2037)	0.34	s	n/d	N/D	n/d	n/d
4-allyl-2-methoxyphenol (eugenol, 4-allylguaiacol)[a]	strongly warm spicy, clove-like	1334 (1338)	2198 (2165)	0.11	w	n/d	N/D	n/d	n/d
methyl *trans*-cinnamate	fruity, balsamic, somewhat strawberry-like	1360 (1361)	2113 (2088)	6.56	s	s	23.28	s	m
vanillin	intensive sweet, tenacious creamy, characteristic vanilla aroma	1368 (1360)	2616 (2586)	sat.	s	s	sat.	s	s
β-damascenone[b,c]	woody, floral, herbal, green and fruity	N/A (1370)	1850 (1821)	N/D	n/d	n/d	0.02	n/d	m
vanillyl methyl ether[a]	sweetish, fruity	1377 (1374)	2346 (2268)	0.15	n/d	m	N/D	n/d	n/d
2,5-dihydroxybenzaldehyde[a]	mild aromatic, somewhat spicy, medicinal	1407 (1420)	3040 (2877)	0.12	n/d	m	N/D	n/d	n/d
trans-cinnamic acid	sweet aromatic, balsamic, somewhat cinnamic-like	1413 (1387)	2907 (2845)	0.17	m	m	1.65	s	n/d
methyl 4-hydroxybenzoate (methylparaben)	sweet aromatic, phenolic, fruity	1429 (1425)	2988 (N/A)	0.38	w	n/d	1.56	s	m

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Table 2. continued

compound[a−c]	descriptor[d]	RI_{DB-1}[e] ($RI_{lib\ apol}$)	RI_{wax}[e] ($RI_{lib\ pol}$)	Bourbon tradit concn[f] (mg/kg)	Bourbon tradit odor strength[g] (DB-1)	Bourbon tradit odor strength[g] (WAX)	Ugandan tradit concn[f] (mg/kg)	Ugandan tradit odor strength[g] (DB-1)	Ugandan tradit odor strength[g] (WAX)
ethyl *trans*-cinnamate	cinnamon	1447 (1445)	2168 (2138)	0.04	n/d	n/d	1.09	n/d	w
acetovanillone (apocynin)	sweet aromatic, somewhat vanilla-like	1469 (1458)	2689 (2640)	1.49	s	m	0.82	s	m
germacrene D	minty, woody, herbal, sweet, hay- and tea-like with dry tobacco nuances	1486 (1496)	1713 (1712)	0.10	n/d	n/d	0.12	m	w
methyl vanillate	sweet aromatic, spicy, slightly vanilla	1496 (1482)	N/A (1457)	0.42	w	n/d	1.18	s	n/d
methyl vanillyl ketone (guaiacylacetone, 4-hydroxy-3-	sweet powdery vanilla creamy balsamic	1507 (1498)	2702 (N/A)	0.04	n/d	n/d	0.06	n/d	m
4-hydroxy-3-methoxybenzoic acid (vanillic acid)	sweet aromatic, somewhat vanilla; creamy, milky	1530 (1518)	N/A (N/A)	0.57	m	n/d	0.49	m	n/d
dodecanoic acid (lauric acid)[a,c]	mild fatty	N/A (1566)	2511 (2491)	0.21	n/d	m	N/D	n/d	n/d
3,5-dimethoxy-4-hydroxybenzaldehyde (syringaldehyde, 5-methoxyvanillin)	sweet aromatic, slightly floral	1618 (1609)	2987 (N/A)	0.17	n/d	n/d	0.35	w	n/d
4-isopropyl-1,6-dimethylnaphthalene (cadalene)[c]	herbal, savory	N/A (1653)	2262 (2200)	0.06	n/d	m	0.11	n/d	m

[a]Odor-active compounds found only in traditionally cured Bourbon bean extract. [b]Odor-active compounds found only in traditionally cured Ugandan bean extract. [c]Compounds are not listed in Table 1 because they were detected only in the polar column. [d]Odor description was determined at the sniffing port and compared with available descriptors in the literature. [e]Retention indices were calibrated using *n*-alkanes. N/A, not available. [f]Concentration: sat., saturated, N/D, not detected. [g]Odor strength: s, strong; m, medium; w, weak; n/d, not detected. The intensity was perceived on the amount at the sniffing port, which was correlated to the concentration in the vanilla beans.

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study, probably lost in the residues in the SAFE distillation due to its nonvolatility (its melting point is 213−217 °C, and it decomposes when it is boiling).[17] Vanillic acid was detected only in Ugandan vanilla beans at 0.48 mg/kg, likely due to the same reason as *p*-hydroxybenzoic acid (its melting point is 208−210 °C, and the boiling point of vanillic acid is not available).[17] *p*-Hydroxybenzaldehyde was reported as 873.3 ppm in the Mexican vanilla bean (*V. planifolia* G. Jackson) quantitated by HPLC.[7] The extract was concentrated, and the authors stated that *p*-hydroxybenzaldehyde could not be quantified by GC-FID due to the saturation of the detector. Hartman et al. reported 1040 ppm *p*-hydroxybenzaldehyde in Bourbon vanilla beans,[10] whereas Adedeji et al. reported 790 and 950 ppm of *p*-hydroxybenzaldehyde in two Madagascan beans.[11] Both groups used the DTD-GC-FID method to quantify the compound from the milled vanilla beans. An average concentration of 0.12 g of *p*-hydroxybenzaldehyde per 100 g of Ugandan vanilla bean was found by Gassenmeier et al. using HPLC.[8] All of these analyses used either the extracts with organic solvents or the milled vanilla beans without further preparation. In this study, *p*-hydroxybenzaldehyde was found at concentrations of 27.06 and 8.61 mg/kg in the Bourbon and Ugandan bean extracts, respectively. The low concentration of this compound was probably due to the loss in the distillation step, maybe because of decomposition during distillation. *p*-Hydroxybenzoic acid, vanillic acid, and *p*-hydroxybenzaldehyde could be quantified using reverse phase high-pressure liquid chromatography (HPLC) or ultrahigh-pressure-liquid-chromatography (UHPLC) with UV detection.[9]

Excluding vanillin, the mass ratio of the total of the volatile compounds was 301 mg/kg for Bourbon and 398 mg/kg for Ugandan vanilla bean extracts. There were 40 compounds in the Bourbon vanilla bean extract that were not detected in the Ugandan vanilla bean extract, whereas there were only 21 compounds in the Ugandan vanilla bean extract that were not detected in the Bourbon vanilla bean extract. This may indicate that not only more abundant compounds but also many trace compounds contribute to the elegant and complicated Bourbon vanilla flavors.

There were 13 nitrogen-containing compounds identified in these two bean extracts, which are labeled as "unidentified" in Table 1 due to the internal interest within the company. Twenty terpenoids were identified in these two bean extracts, including 5 monoterpenoids, 14 sesquiterpenoids, and 1 diterpenoid. The sesquiterpenoids were detected in the gas chromatograms in the region of 40−46 min, right after the vanillin peak in the nonpolar GC chromatogram. Propylene glycol was found in the analysis of the volatiles of vanilla bean extracts, and it could be the result of some sort of contamination during packaging, extraction, distillation, or GC injection. Propylene glycol derivatives, including 1,1′-dipropylene glycol 2′-methyl ether and vanillin acetals, were likely formed in the GC injection port. There were also three phthalates identified, including diethyl phthalate and dibutyl phthalate, which were reported by Klimes and Lamparsky.[4] It is not certain whether dioctyl phthalate, a common plasticizer, is present due to contamination.

The organoleptic evaluation of the distilled extracts of traditionally cured Bourbon and Ugandan vanilla beans in dichloromethane was performed by a flavorist in the company, using paper blotters. The Bourbon bean extract was described as "sweet, beany-phenolic, woody, some chocolate note, slightly smoky (guaiacol)", whereas the Ugandan bean extract was described as "sweet, powdery, balsamic, beany-phenolic, more anisic compounds, cinnamic". To identify and analyze the odor-active components in the distilled vanilla extracts, the extracts were subjected to GC-O analysis. As described under Materials and Methods, effluent coming out of the column was split into three detectors, namely, MSD, FPD, and GC-O sniffing port, via a cross splitter. The synchronicity of the peaks from the detectors was confirmed by a model mixture comprising a few known odorants. Then the identification of odor-active compounds in the vanilla bean extracts was based on the panelists' descriptions of the effluents at the sniffing port in comparison with the available descriptors in the Flavor Raw Materials database and other aroma descriptions from the literature, in addition to retention indices (both apolar and polar) and mass spectra. Most of the descriptions by the panelists were consistent with the literature. However, 1-hexanol was described as "roasty, nutty, pleasant cheesy" by the panelists and may be perceived as fruity and winey rather than green.

In total, 78 odorants were identified in the traditionally cured Bourbon and Ugandan vanilla bean extracts using both apolar and polar columns. The odorants are listed in Table 2, among which 10 were confirmed with authentic compounds and the others were tentatively identified on the basis of the above criteria. Of the odorants, 9 compounds were found only in the Bourbon vanilla beans, and 2 were unique to the Ugandan vanilla beans. The two compounds only found in the traditionally cured Ugandan vanilla beans were 5-isopropyl-2-methylphenol (carvacrol) and β-damascenone. There are four compounds in Table 2 that are not listed in Table 1, including 5-isopropyl-2-methylphenol (carvacrol), β-damascenone, dodecanoic acid (lauric acid), and 4-isopropyl-1,6-dimethylnaphthalene (cadalene), because they were found only using the polar column. Compounds listed in Table 1 were identified with a nonpolar column.

Among the 78 odorants identified in the traditionally cured Bourbon and Ugandan vanilla beans, vanillin was the most abundant followed by guaiacol. The two isomers of 2,3-butanediol were within the range of 10−50 mg/kg. There were 11 and 40 compounds detected as odor-active within the mass ratio ranges of 1−10 and 0.1−1 mg/kg, respectively. Twenty-three compounds, including 3,5-octadien-2-one, 1-nonanol, γ-octalactone, 4-allylphenol, heliotropine, methyl decanoate, β-damascenone, and some nitrogen-containing compounds, were evaluated as the most powerful and rated medium or strong intensity at a mass ratio $\leq$0.05 mg/kg.

Interestingly, of the six more concentrated strong odorants, namely, acetic acid, methyl salicylate, *p*-anisaldehyde, methyl *trans*-cinnamate, *trans*-cinnamic acid, and methylparaben, five were more abundant in the Ugandan vanilla beans, with *p*-anisaldehyde as the most different in terms of concentration, 14 times more in the Ugandan vanilla beans, and methyl *trans*-cinnamate as the most concentrated (23 mg/kg in the Ugandan vanilla beans) strong odorant. Methyl salicylate was about 3 times more concentrated in the Bourbon vanilla beans. Among the more concentrated medium-intensity odorants, methyl *cis*-cinnamate was the most different in terms of concentration between the two vanilla bean extracts, at about 7 times more in the Ugandan vanilla beans. Among the weak-intensity odorants, ethyl *trans*-cinnamate concentrations were the most different between the two vanilla bean extracts, with 25 times more present in the Ugandan vanilla bean extracts. These analytical

results were consistent with the olfactive evaluation of the distilled vanilla bean extracts.

In conclusion, 246 compounds have been identified in the extracts of the traditionally cured Bourbon and Ugandan vanilla beans, of which 78 were listed as odor-active compounds. There were substantial analytical differences in the odor-active compounds of the two extracts. It needs to be pointed out that the results in this study are based on only one crop of Bourbon and Ugandan vanilla beans. The knowledge on vanilla would be more comprehensive if there were research on variances between different crops from different seasons in a year, different years, and different locations in the same region.

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Notes

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