Gas Chromatography/Olfactory Analysis of Lychee (*Litchi chinesis* Sonn.)

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Volatile compounds from lychee (*Litchi chinesis* Sonn.), a tropical fruit native to China, were extracted using both Freon 113 and ethyl acetate solvents. The odor-active compounds present in the fruit were isolated and characterized using gas chromatography/olfactory (GC/O), chromatography, and mass spectrometry. Authentic standards were used to determine mass spectral, retention index, and odor match. GC/O analysis detected at least 60 odor-active volatiles in the fruit extract. More odor-active volatiles were detected in the ethyl acetate extract than in the nonpolar Freon extract. Among the compounds that had significant odor activity, geraniol, guaiacol, vanillin, 2-acetyl-2-thiazoline, 2-phenylethanol, unknown (**58**), (*Z*)-2-nonenal, *β*-damascenone, 1-octen-3-ol, Furaneol, and linalool were found to be the most odor-active. On the basis of their calculated odor activity values (OAV), isobutyl acetate, guaiacol, *cis*-rose oxide, 2-acetyl-2-thiazoline, *β*-damascenone, Furaneol, linalool, (*E*)-2-nonenal, geraniol, and isovaleric acid were determined to significantly contribute to the aroma of this fruit. GC/O analysis confirmed that 2-phenylethanol was probably responsible for the floral character and that the citrus-fruity aroma is due to the presence of many odor-active terpenes, particularly geraniol. Although *cis*-rose oxide was only 30% as active in GC/O as the most potent odor, its high OAV indicates its importance to the character of lychee odor. An unknown sesquiterpene-like compound (**58**), with a lychee-like odor characteristic of the fresh fruit, was identified by GC/O as being a highly odor potent compound. Taken together, the aroma of lychee was determined to be due to the interaction between compounds with floral, nutty, citrus, and fruity aromas.

Keywords: *Aroma; GC/O; lychee; volatiles*

INTRODUCTION

The lychee is a subtropical fruit native to China and belongs in the same family (Sapindaceae) as the tropical fruits longan and rambutan. Its aroma is often described as being rose-floral and citrus-like. Its commercial importance and popularity among consumers has continued to expand to markets outside Asia, where this fruit is most popular. In North America, trade in fresh lychee has shown a steady increase, and demand for lychees has crossed from Asian communities to mainstream markets (Klotzbach, 1995). Availability of the fresh fruit is limited to the summer months, because of its short production period and shelf life. However, there is a global demand for the processed fruit exported mainly from China, Taiwan, and Thailand.

Previous studies have identified the volatile components in this fruit. Johnston et al. (1980) were the first to report on the volatiles of lychee, suggesting the citrus flavor was due to the presence of compounds such as geranial and neral and its floral note was due mainly to 2-phenylethanol. Two other papers, subsequently published, reported headspace and neutral volatiles in this fruit (Toulemonde and Beauverd, 1985; Froehlich and Schreier, 1986). Among the volatiles identified, limonene, rose oxide, nonanal, decanal, citronellol, and geraniol were considered to be significant for the fruity-floral and citrus notes of the fruit. However, no gas chromatography/olfactometry (GC/O) studies have been performed to date to indicate the relative odor potency of the volatiles identified. In this paper we describe the identification and quantification of the most potent odorants extracted from lychee fruit as detected by GC/O.

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MATERIALS AND METHODS

Materials. (*E*)-4,5-Epoxy-(*E*)-2-decenal was synthesized according to the method of Ong et al. (1998). (*E*)-Furan linalool oxide was a gift from San Ei Gen, Inc. (Osaka, Japan). All other authentic standards were obtained commercially.

Sample Preparation. Fruits (cv. No Mai Chi) obtained from China through a wholesaler were peeled and pitted. Two batches of 1.5 kg of the flesh were blended with 1.0 M $CaCl_2$ for 1 min to inactivate enzymes (Shure, 1992), yielding 1.4 L of the juice. The juice was sequentially extracted with Freon 113 and ethyl acetate. Both extracts were dried with anhydrous magnesium sulfate and serially diluted or concentrated 3-fold with the respective solvents. The most concentrated extract was 243-fold and the least $^1/_{27}$-fold.

GC/O. Extracted samples were analyzed by a single sniffer on a GC/O system (CharmAnalysis) (Datu, Inc., Geneva, NY) equipped with either an HP-1 (15 m × 0.32 mm) or an HP-Innowax (15 m × 0.32 mm) column (Acree et al., 1984). The GC effluent was combined with an olfactometer airstream at 7 L min^{-1} and 1 cm in diameter. The oven temperature was programmed from 35 to 250 °C at 6 °C/min. Prior to sniffing the samples, the subject was screened for olfactory acuity according to a training procedure described by Marin et al. (1988). The sniffer was able to detect 0.82 ng of ethyl butyrate, 0.99 ng of ethyl hexanoate, 0.82 ng of 1,8-cineole, 1.20 ng of carvone, 0.046 ng of *β*-damascenone, and 0.41 ng of *o*-aminoacetophenone eluting from the GC/O. All extract dilu-

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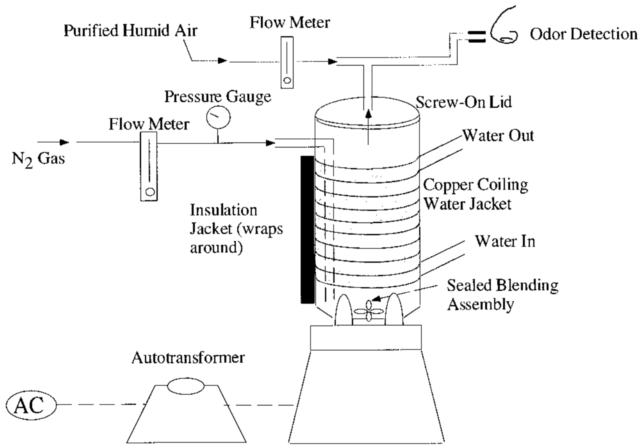

Figure 1. Diagram of a modified RAS for odor threshold determination.

tions were sniffed twice (repeated measure) until no odor was observed (detection threshold), and the retention time of each odorant was converted to Kovats indices using 7−28-carbon normal paraffins. Caramel, citrus, earthy, floral, fruity, green, medicine, musty, nutty, rancid, urine, and woody were the words used to describe the most potent odorants chosen from a lexicon that also included the words burnt, buttery, coconut, fatty, grassy, plastic, spicy, and sweaty. The lexicon was developed by sniffing the 243-fold sample three times and selecting the 20 most frequently used words.

Quantitative Analyses. The most odor-active volatiles in lychee as determined by GC/O were quantified using GC mass fragmentography of the most abundant ion. The concentration of each volatile was adjusted for loss during extraction by determining the percent recovery of each compound in a model system (Table 3). Odor activity values (OAVs) were determined by dividing the concentration by its odor detection threshold (Guadagni et al., 1966).

Determination of Threshold Values. The odor threshold values for the most odor-active compounds as determined by GC/O analysis were determined using a modified retronasal aroma stimulator (RAS) as shown in Figure 1. The RAS was originally developed for use as a device that stimulated the release of aroma compounds in the mouth (Roberts, 1996). Known amounts of a flavor compound were added in increasing concentrations to a mixture consisting of 50 mL of artificial saliva (20 mmol/L $NaHCO_3$, 2.75 mmol/L K_2HPO_4, 12.2 mmol/L KH_2PO_4, and 15 mmol/L NaCl with a pH of 7.0) and 150 mL of a fruit juice matrix (15 °Brix sucrose and 0.08% citric acid in distilled water). The mixture was maintained at 37 °C and sheared at 330 s^{-1} for 30 s to stimulate gustation in the mouth. A stream of nitrogen flowed through the mixture at 20 mL s^{-1}, and the eluting air was diverted into a sniff tube for odor detection. All threshold values were determined in micrograms per liter units.

Capillary Gas Chromatography/Mass Spectrometry (GC/MS). An HP model 5985 MSD GC/MS was used with an HP-1 (25 m × 0.32 mm) or an HP-Innowax (25 m × 0.32 mm) column. The oven temperature was programmed from 35 to 250 °C at 4 °C/min. Retention indices (RI) of standard compounds that matched with RI of unknowns detected by GC/O were tentatively identified. Confirmation of unknowns was based upon odor and mass spectral and RI matches with authentic standards.

RESULTS AND DISCUSSION

GC/O analysis detected ∼60 odor-active volatiles in the fruit extract. Figure 2 shows the combined odor spectrum for both Freon and ethyl acetate extracts, while Tables 1 and 2 list the odorants that were detected in the fruit by GC/O analysis. The tables include Charm values (Acree, 1997) and odor spectrum values (OSV) (Ong et al., 1998). OSVs are independent of concentration and approximate the relative importance of component odorants, while Charm and OAVs as true activity measures are linear functions of concentration. Among the compounds that had significant odor activity as indicated by their OSVs, geraniol, guaiacol, vanillin, 2-acetyl-2-thiazoline, 2-phenylethanol, an unknown (**58**), β-damascenone, 1-octen-3-ol, (*Z*)-2-nonenal, and Furaneol were identified as being the most odor potent compounds (Table 1). Other compounds identified that had significantly high OSVs included linalool, hydrocinnamic acid, isobutyl acetate, (*E*)-4,5-epoxy-(*E*)-2-decenal, isoborneol, isovaleric acid, (*E*)-furan linalool oxide, (*E*)-2-nonenal, phenylacetic acid, and γ-nonalactone.

The remaining potent odorants (below 40% OSV) in lychee as detected by GC/O are presented in Table 2. Of the 34 compounds detected, 22 were identified by GC/O and/or GC/MS and the remaining were not identified. Except for *cis*-rose oxide, these compounds should be tested for their contribution to the complexity of lychee flavor.

More odor-active volatiles were detected in the ethyl acetate extract than in the nonpolar Freon extract. However, as shown in Tables 1 and 2, some compounds are more soluble in Freon, and combining the results of the Charm values would provide a more accurate representation of the sample.

Among the most odor potent compounds detected (Table 1), guaiacol, 2-acetyl-2-thiazoline, β-damascenone, (*E*)-4,5-epoxy-(*E*)-2-decenal, and γ-nonalactone were not previously identified in lychee. It is interesting to note that all of these compounds have low odor threshold values, suggesting that they may have been present at a concentration too low to have been detected. GC/O analysis confirmed that 2-phenylethanol, *cis*-rose oxide, and phenethyl acetate were responsible for the rose-floral notes described previously by Johnston et al. (1980) as being characteristic of lychees. Also, the presence of many odor-active terpenes, particularly geraniol, was determined to contribute to the citrus-fruity aroma. As reported previously (Toulemonde and Beauverd, 1985), isomers of caryophyllene, humulene, and guaiene were also found in the extracts analyzed. However, they contributed no odor activity at the GC/O. Although *cis*-rose oxide was only 30% as active in GC/O as the most potent odor, we believe that it is essential to the character of lychee odor. This was further substantiated by determination of the OAV for this compound.

Table 3 lists the concentrations, odor threshold values, and calculated OAVs for the 22 most odor potent compounds determined by GC/O analysis. Determination of the threshold values using the RAS provided a more accurate approximation of actual flavor release in the mouth and, as such, a more meaningful representation of each compound's OAV. Presently, most threshold values found in the literature are determined in air, water, or various solvent mixtures and detected either orthonasally or retronasally (Guth and Grosch, 1990; Fazzalari, 1978; Stahl, 1973). However, the RAS provided the added advantage of allowing the determination of each compound to be made in the actual food matrix being studied within a controlled environment while being less time-consuming. On the basis of the concentrations and threshold values determined, the OAV for each volatile was calculated. On the basis of

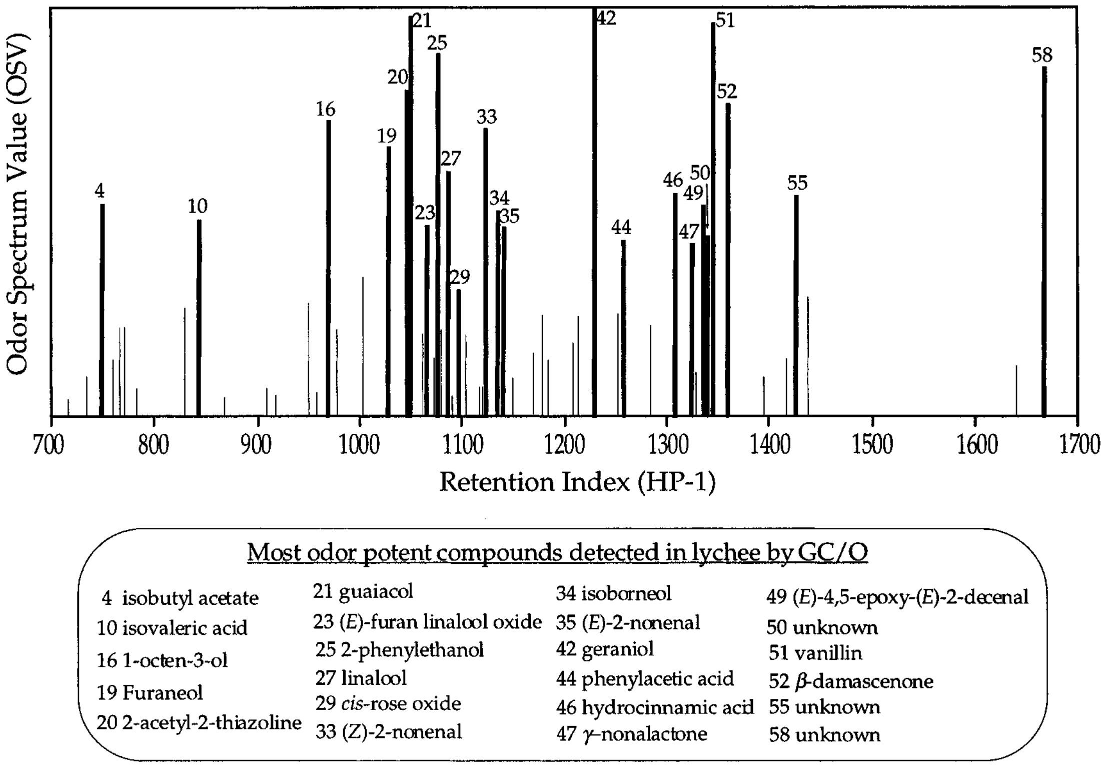

Figure 2. Odor spectrum of lychee fruit—combined Freon and ethyl acetate extracts.

Table 1. Most Potent Odorants Found in the Extracts of Lychee Fruit (above 40% OSV[a])

peak no.	compd detected	CAS Registry No.	extract[b]	retention indices HP-1	retention indices HP-Innowax	descriptors	Charm values[c]	OSV[a]	confirmed by GC/O	confirmed by MS
42	geraniol	106-24-1	EAC	1230	1850	fruity, floral	27431	100	+	+
21	guaiacol	90-05-1	EAC	1056	1848	medicine	26937	99	+	+
51	vanillin	121-33	EAC/FR	1345	2591	vanilla	25666	97	+	+
20	2-acetyl-2-thiazoline	29926-41-8	EAC	1055	1725	nutty	22266	90	+	+
25	2-phenylethanol	60-12-8	EAC	1078	1905	floral	21085	88	+	+
58	unknown		FR	1670	2230	lychee	19016	83	−	−
52	*β*-damascenone	23726-93-4	FR	1356	1790	fruity, floral	16329	77	+	+
16	1-octen-3-ol	3391-86-4	EAC	958	1445	earthy	13698	71	+	+
33	(*Z*)-2-nonenal	60784-31-8	EAC/FR	1121	1492	plastic, green	13011	69	+	+
19	Furaneol	3658-77-3	EAC	1029	2020	caramel	11688	65	+	+
27	linalool	78-70-6	EAC	1083	1548	citrus green	9569	59	+	+
46	hydrocinnamic acid	501-52-0	EAC	1304	2650	balsamic	8293	55	+	+
55	unknown		EAC/FR	1428		woody	7707	53	−	−
4	isobutyl acetate	110-19-0	EAC	760	1009	fruity	7171	51	+	+
49	(*E*)-4,5-epoxy-(*E*)-2-decenal		EAC/FR	1335	1913	woody	6964	50	+	+
34	isoborneol	124-76-5	EAC/FR	1128	1664	earthy	6589	49	+	+
10	isovaleric acid	503-74-2	EAC	840	1660	rancid	6097	47	+	+
23	(*E*)-furan linalool oxide	34995-77-2	EAC	1065	1453	green	5718	46	+	+
35	(*E*)-2-nonenal	18829-56-6	EAC/FR	1130	1519	plastic	5391	44	+	+
50	unknown		EAC/FR	1338		woody	5006	43	−	−
44	phenylacetic acid	103-82-2	EAC	1236	2568	urine	4889	42	+	+
47	*γ*-nonalactone	104-61-0	EAC	1308	2008	musty	4576	41	+	+

[a] Odor spectrum value (OSV) is the normalized Charm value modified with an approximate Stevens' law exponent ($n = 0.5$). [b] Extract in which most compound was detected: Freon 113 (FR); ethyl acetate (EAC). [c] Charm values were the sum total of both Freon and ethyl acetate extracts.

OAVs, isobutyl acetate, guaiacol, *cis*-rose oxide, 2-acetyl-2-thiazoline, *β*-damascenone, Furaneol, linalool, (*E*)-2-nonenal, geraniol, and isovaleric acid had values >10, indicating a significant contribution to the aroma of this fruit. Compounds such as 2-phenylethanol, 1-octen-3-ol, hydrocinnamic acid, and phenylacetic acid had relatively low OAVs (<1), suggesting that this was likely due to their high odor threshold values and/or low volatility in the fruit juice matrix.

Compound **58** was determined by GC/O to have a characteristic lychee-like odor, particularly reminiscent of a freshly peeled fruit. An informal sensory panel provided various odor descriptors for this compound that included woody, citrus, smoky, and sulfury. This compound had an unusually high RI on a nonpolar HP-1

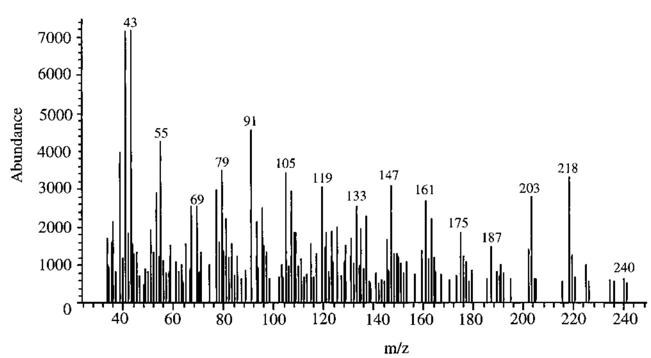

Figure 3. Mass spectrum (EI) of an unknown (**58**) with a lychee-like aroma.

Table 2. Additional Potent Odorants Detected in the Extracts of Lychee Fruit (below 40% OSV[a])

compd detected	CAS Registry No.	extract[b]	retention indices HP-1	retention indices HP-Innowax	descriptors	Charm values[c]	OSV[a]	confirmed by GC/O	confirmed by MS
hexanoic acid	142-62-1	EAC	1005	1836	sweaty	3403	35	+	+
δ-decalactone	705-86-2	EAC	1440	2160	coconut	3013	33	+	+
cis-rose oxide	876-17-5	FR	1092	1338	green, floral	2274	29	+	+
phenethyl acetate	103-45-7	EAC/FR	1233	1803	rose, floral	2096	28	+	+
α-terpineol	10482-56-1	FR	1172	1685	floral	1850	26	−	−
citronellol	7540-51-4	EAC/FR	1212	1764	citrus	1665	25	+	+
ethyl 2-methylbutyrate	7452-79-1	FR	837	1041	fruity	1660	25	+	+
ethyl isohexanoate[d]	25415-67-2	EAC	951	1181	fruity	1629	24	−	+
nonanal	124-19-6	EAC/FR	1081	1380	plastic, fatty	1382	22	+	+
ethyl methacrylate[d]	97-63-2	EAC	775		grassy	1319	22	−	+
hexanal	66-25-1	EAC/FR	780	1071	grassy	1293	22	+	+
octanal	124-13-0	EAC	981	1272	fatty, citrus	1249	21	+	+
(*E*,*E*)-2,4-decadienal	25152-84-5	EAC/FR	1284	1778	citrus, fatty	1173	21	+	+
5M67DH5CP[e]	23747-48-0	EAC	1103	1594	nutty	1116	20	+	+
unknown		EAC	1064		spicy	1068	20	−	−
unknown		EAC/FR	1145		burnt	1016	19	−	−
nerol	106-52-2	EAC	1210	1796	citrus	929	18	+	+
2-methyl-2-butenal[d]	497-03-0	FR	752		green	757	17	−	+
ethyl isobutyrate	97-62-1	EAC	767	984	fruity	684	16	+	+
γ-decalactone	706-14-9	EAC	1418	2109	woody	678	16	+	−
terpinolene	586-62-9	FR	1077	1269	plastic	605	15	+	+
terpinen-4-ol	562-74-3	FR	1160	1621	woody, musty	536	14	+	+
unknown		EAC	1176		musty	497	13	+	+
unknown		FR	1640		woody	470	13	−	−
unknown		EAC	1119		fruity	306	11	−	−
unknown		EAC	1323		woody	263	10	−	−
cinnamic acid	140-10-3	EAC	1394	2852	woody	223	10	+	+
2,6-nonadienal	557-48-2	EAC/FR	1117	1553	green	200	9	+	+
unknown		FR	908		musty	198	9	−	−
unknown		EAC	1087		sweaty	144	7	−	−
isoamyl acetate	123-92-2	FR	860	1101	fruity	141	7	+	+
butyric acid	107-92-6	EAC		1630	rancid	134	7	+	+
unknown		EAC	920		woody	128	7	−	−
(*E*)-2-hexenal	6728-26-3	FR	832	1207	green	100	6	+	+

[a] Odor spectrum value (OSV) is the normalized Charm value modified with an approximate Stevens' law exponent ($n = 0.5$). [b] Extract in which most compound was detected: Freon 113 (FR); ethyl acetate (EAC). [c] Charm values were the sum total of both Freon and ethyl acetate extracts. [d] Tentatively identified by GC/MS. [e] 5-Methyl-6,7-dihydro-5(*H*)cyclopenta(*b*)pyrazine.

Table 3. Concentrations, Odor Thresholds, and OAVs of Most Potent Odorants As Detected by GC/O in Lychee Fruits

peak no.	compd	% recovery[a]	concn[b] (μg/L of juice)	threshold value[c] (ppb)	OAV[d]
42	geraniol	92	51.40	5	10
21	guaiacol	109	179.34	2	90
51	vanillin	112	63.90	1200	0.05
20	2-acetyl-2-thiazoline	97	40.91	0.5	82
25	2-phenylethanol	95	925.40	2000	0.46
52	β-damascenone	100	0.72	0.01	72
16	1-octen-3-ol	79	14.30	18	0.79
33	(*Z*)-2-nonenal		nd[e]		
19	Furaneol	104	1025.60	25	41
27	linalool	100	16.32	1.5	11
46	hydrocinnamic acid	104	923.60	5000	0.18
4	isobutyl acetate	85	2120.70	10	210
49	(*E*)-4,5-epoxy-(*E*)-2-decenal	97	15.60	5	3.1
34	isoborneol	100	47.74	10	4.8
10	isovaleric acid	102	123.80	12	10
23	(*E*)-furan linalool oxide	100	3.50	60	0.06
35	(*E*)-2-nonenal	95	3.60	0.1	36
44	phenylacetic acid	104	361.70	2000	0.18
47	γ-nonalactone	115	5.02	2	2.5
29	*cis*-rose oxide	102	8.50	0.1	85

[a] Percent recovery based on the combined recovery of Freon and EAC extracts. [b] Concentration of combined Freon and ethyl acetate extracts. [c] Determined using a modified RAS. [d] OAVs were calculated by dividing the concentration by its odor threshold. Values were rounded to two significant figures. [e] nd, below detection threshold.

column (RI = 1670) and was present at an extremely low concentration in the extracts, making detection by GC/FID or GC/MS difficult. Mass spectral data of this compound tentatively suggest it to be a sesquiterpene-type compound (Figure 3).

Taken together, the aroma of lychee was determined by GC/O to be due to the interaction between compounds with floral (*cis*-rose oxide, 2-phenylethanol), citrus-fruity (geraniol, β-damascenone, linalool, isobutyl acetate), nutty-woody (2-acetyl-2-thiazoline, guaiacol, γ-nonalactone), plastic-green (2-nonenal, linalool oxide), and sweet (Furaneol) aromas with phenylacetic, isovaleric, and hydrocinnamic acids providing complexity to its aroma.

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