

STRUCTURE AND REPRESENTATION OF AROMATIC COMPOUNDS OF GRAPE BRANDY PRODUCED FROM MUSCAT TABLE GRAPEVINE (*VITIS VINIFERA L.*) CULTIVARS

SAŠA MATIJAŠEVIĆ¹, ZORAN BEŠLIĆ¹, ZORAN PRŽIĆ¹, ALEKSANDAR PETROVIĆ¹, ZORICA RANKOVIĆ-VASIĆ¹, BRATISLAV ĆIRKOVIĆ², DRAGOSLAV IVANIŠEVIĆ³, DUŠICA ĆIRKOVIĆ⁴, MERSIJA DELIĆ⁵

¹Faculty of Agriculture, University of Belgrade, Serbia

² Faculty of Agriculture, University of Priština-Kosovska Mitrovica, Serbia

³ Faculty of Agriculture, University of Novi Sad, Serbia

⁴ College of Agriculture and Food technology, Prokuplje, Serbia

⁵Faculty of Agriculture and Food Sciences, University of Sarajevo, Bosnia and Herzegovina

ABSTRACT

A combined gas chromatographic-mass spectrometric (GC/MS) method was used in this study to detect volatile components of eight samples of grape brandy produced from Muscat table grapevine (*Vitis vinifera L.*) cultivars. The gas chromatographic-mass spectrometric analysis of the extracts resulted in the identification of 155 components including

64 esters, 35 terpenes, 17 acids, 8 alcohols, 3 aldehydes, 8 ketones, 14 hydrocarbons (alkanes, alkenes and alkenols), 5 acetals and 1 heptanoic acid anhydride. Ethyl esters of C₈ – C₁₈ fatty acids and terpenic compounds were considerably more abundant in all grape brandy samples as compared to the other volatile compounds identified.

Key words: grape brandy, aroma, GC/MS, ethyl esters, terpenes, higher alcohols

INTRODUCTION

Grape brandy is obtained through fermentation and distillation of the whole non-strained mash of noble grape *Vitis vinifera L.* cultivars. A beverage similar but not identical to grape brandy, the so-called Pisco (obtained by distillation of wine), is produced in some countries of South America (Chile, Peru and Argentina) as well as in Italy where it is marketed under the name L'aquavite d'uva.

Grape brandy quality is dependent upon a number of factors, most notably cultivar-specific characteristics, grape processing method, alcoholic fermentation and distillation method (Versini et al., 1993, Nikicevic et al.,

2000, Wondra and Berovic, 2001, Radeka et al., 2008). The aromatic potential of different grape cultivars is of particular importance for grape brandy quality. As regards Muscat cultivars, this potential arises from the terpenic content Agosin et al.(2000).

Apart from water and ethanol as the main constituents, grape brandy also contains a number of other components the concentration of which is mostly dependent upon the cultivar i.e. raw materials used and the technology employed (fermentation method, distillation process, etc.).

The aroma of a grape product is the result of simultaneous activities of a large number of aromatic substances. Some grape products require the presence of few compounds that give them their cultivar-typical aroma, whereas some others have their distinctive character generated by only a wide range of aromatic substances occurring at a particular ratio. Generally, wines contain 10^{-4} to 10^{-11} g/L of certain aromatic substances Rapp (1989). The odour detection threshold of some aromatic substances is much more important than their abundance. In sensorial terms, much higher significance is attributed to odour-active substances that show a low odour detection threshold and that, despite their lower percentage, play a considerably more important role than the components of low odour intensity present at higher concentrations.

Higher alcohols are quantitatively the largest group of volatile compounds found in distillates, giving them their distinctive aroma, flavour and fundamental character Soufleros et al. (2004). The most important aroma factors in Muscat and non-Muscat cultivars are terpenic and aliphatic alcohols, respectively (Gomez et al., 1994, Gunata et al., 1986).

Free fatty acids are common components of distilled alcoholic beverages primarily generated through carbohydrate metabolism by yeasts. Fatty acids are associated with a numerous group of aroma factors including esters among others Luiz Silva et al. (1996).

Esters make a significant contribution to distillate flavour by producing pleasant fruity and floral aromas that serve as an indicator of beverage quality (Soufleros et al., 2004, Hernández-Gómez et al.,

2005). Esters are produced by yeasts during alcoholic fermentation, i.e. during reactions between alcohol and acetyl-CoA. Given the fact that ethanol is the most abundant alcohol in wine, ethyl acetate is the major ester formed during fermentation Mamede et al.(2005). It is of high importance for distillate quality, as regards its unpleasant aroma Luiz Silva et al. (1996).

Aldehydes can be found in distilled beverages. They are considered indicators of spontaneous oxidation or activity of undesirable contaminating bacteria Luiz Silva et al. (1996).

The objective of this study was to evaluate the effect of grape cultivar on the composition and structure of the aromatic complex as well as the relative content of certain volatile compounds that contribute to the aromatic profile of the grape brandy produced from the following Muscat table grapevine cultivars: Demir Kapija (sample I), Early Muscat (sample II), Radmilovac Muscat (sample III), Banat Muscat (sample IV), Black Muscat (sample V), Smederevo Muscat (sample VI), Italia (sample VII) and Dattier (sampleVIII).

MATERIALS AND METHODS

Grape brandy making technology

The brandy making technological process was unified and implemented as follows: grapes were harvested fully ripe (grape ripeness was determined through

monitoring of the sugar accumulation dynamics). A sample of 10 kg of grapes was collected from each cultivar. Harvest was followed by grape disintegration (pressing) and stem separation. Fermentation was performed in 20 l

plastic containers using standard procedure, i.e. within the autochthonous microflora without sulphuring. Fermentation was carried out at a temperature of 20 °C with the cap immersed. After alcoholic fermentation, the fermented mash was distilled using a simple brass Charente-type device. The fermented mash was distilled without separating the first brandy, in order to provide maximum transfer of aromatic ingredients to the raw distillate. Soft grape brandies were produced by distillation. They were also re-distilled using a 5 l Charente-type device in order to produce double-distilled brandy. During the second distillation, the first distillate fraction was separated at the amount of 1 % of the initial quantity of the raw distillate. Accumulation of the middle fraction was carried out until the average concentration (in the mass) decreased to a minimum of 65 % vol.

The distilled grape brandies produced were subjected to gradual harmonisation for 3 weeks, followed by gradual adjustment or dilution to reach the final alcoholic strength of 45 % vol.

GC and GC/MS analysis of volatile compounds

Liquid-liquid solvent extraction was used to prepare aroma extracts. All samples analysed were submitted to pentane extraction involving the use of 100 ml brandy and 1 ml pentane for each sample. After 3 minutes of mixing, the sample-containing flask was refrigerated to remove the pentane phase.

Gas chromatographic analysis was performed using a HP 5890 gas

chromatograph equipped with a flame ionisation detector (FID) and a split/splitless injector. The separation was achieved using a HP-5 (5 % diphenyl and 95 % dimethylpolysiloxane) fused silica capillary column, 30 m x 0.25 mm i.d., 0.25 µm film thickness. GC oven temperature was programmed from 50 °C (6 min.) to 285 °C at a rate of 4.3 °C / min. Hydrogen was used as the carrier gas; the flow rate was 1.0 mL / min at 210 °C. The injector temperature was 250 °C, detector temperature 280 °C, and the injection mode splitless. An injection volume of 1.0 µL was used for the beverage extract.

Gas chromatographic-mass spectrometric (GC / MS) analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD), in positive ion electron impact (EI) mode. The separation was achieved using an Agilent 19091S-433 HP-5MS fused silica capillary column, 30 m x 0.25 mm i.d., 0.25 µm film thickness. GC oven temperature was programmed from 60 °C to 285 °C at a rate of 4.3 °C / min. Helium was used as the carrier gas, inlet pressure: 25 kPa, linear velocity: 1 mL / min., at 210 °C. Injector temperature was 250 °C, and the injection mode splitless. MS scan conditions: source temperature, 200 °C; interface temperature, 250 °C; E energy, 70 eV; mass scan range, 40-350 amu (atomic mass units). Component identification was performed using both the retention index and comparison with reference spectra (Wiley database). The (relative) percentage of the compounds identified was computed from the GC peak area.

RESULTS AND DISCUSSIONS

The volatile components identified in eight samples of grape brandies are

presented in Table 1. The individual samples (I, II, III, IV, V, VI, VII and VIII)

were found to contain a total of 66, 76, 77, 62, 63, 62, 67 and 27 free aromatic compounds, respectively. The components identified belonged to different groups of compounds including alcohols, esters, terpenes, acids, aldehydes, ketones, acetals and hydrocarbons.

Table 1 shows that dodecanoic acid has the highest relative value in all distillate samples analysed as compared to the other fatty acids. Moreover, dodecanoic acid was identified in all grape brandy samples (I – VIII). The relative content of dodecanoic acid ranged from 0.83% (sample III) to 2.30% (sample VII). Decanoic, hexanoic and octanoic fatty acids mostly impart unpleasant odours of rancid fat, greasy oils, lard or spoiled cheese (Genovese et al., 2004, Ferreira et al., 2002, Rogerson and De Freitas, 2002).

Results on the aromatic components identified in this study (Table 1) show that ethyl esters of C₈ – C₁₈ fatty acids were the most numerous and most abundant in all samples, with ethyl decanoate (3.29% sample I – 30.57% sample VIII) and ethyl hexadecanoate (5.81% sample VI – 18.10% sample II) having the highest abundance. Apart from them, the samples had a significant relative content of ethyl 9-hexadecanoate, ethyl dodecanoate, ethyl octanoate, ethyl linoleate and ethyl tetradecanoate.

The relative content of ethyl octanoate, ethyl decanoate and ethyl dodecanoate was higher in grape brandies produced from cvs. Black Muscat, Smederevo Muscat, Italia and Dattier (samples V through VIII) than in those from cvs. Demir Kapija, Early Muscat, Radmilovac Muscat and Banat Muscat (samples I through IV) predominated by ethyl hexadecanoate and ethyl 9-

hexadecanoate. Fatty acid esters largely contribute to the pleasant fruity and floral aroma of the distillate (Soufleros et al., 2004, Hernández-Gómez et al., 2005). Ethyl octanoate imparts a pleasant fresh fruity aroma Ferreira et al., (2002). Ethyl hexanoate produces a tropical fruit odour and aroma, whereas ethyl octanoate and ethyl dodecanoate give a pear-like aroma and a characteristic fruity aroma, respectively Rogerson and De Freitas (2002). This author Genovese et al.(2004) relates the fruity sweet aroma suggestive of bananas and apples to ethyl butanoate; a vinous, apple- and banana-like aroma to ethyl hexanoate; a banana-, pineapple- and brandy-like aroma to ethyl octanoate; a brandy, oily, fruity and grape-like aroma to ethyl decanoate; lard- and soap-like odour to both ethyl dodecanoate and ethyl tetradecanoate.

Isoamyl acetate, linalyl acetate, geranyl acetate, citronellyl acetate and neryl acetate comprise a group of acetic acid esters. Their abundance in the distillates was lower than that of the ethyl esters of fatty acids. Isoamyl acetate and citronellyl acetate were identified in all grape brandy samples apart from the brandy produced from cv. Dattier (sample VIII).

As for the terpenic content (Table 1), the most abundant components include limonene (1.00% sample VIII – 8.70% sample III), γ -terpinene (0.16% sample VIII – 1.72% sample III) and linalool (0.45% sample VII – 3.03% sample VI) identified in the distillates of all test cultivars. Apart from these compounds, farnesol was identified in all grape brandy samples (I through VIII), at a considerably lower relative content. Apart from the above compounds, the following components were also identified in most grape brandy samples: α -terpinolene, hotrienol, citronellol, manoil oxide, myrcene, α -terpinene and *p*-cimene.

The relative content of limonene, γ -terpinene, linalool and citronelol was higher in the grape brandy made from cvs. Demir Kapija, Early Muscat, Radmilovac Muscat and Banat Muscat (samples I through IV) than in those produced from cvs. Black Muscat, Smederevo Muscat, Italia and Dattier (samples V through VIII). Terpenes are mostly responsible for fine aromatic, flowery and floral aromas Fang et al. (2006). Linalool and citronelol play the most important role among terpenols in that they significantly contribute to the aroma, generating the aroma of roses, anise seed, grapefruit, green lemon and citrus. Limonene enhances the fruity aroma with a hint of citrus, α -terpineol gives the aroma of flowers, iris and pine wood. Geraniol can also produce the aroma of flowers, rose in particular Diéguez et al. (2003). The aromatic compounds found in trace amounts in grape brandies such as α -terpinolene, hotrienol, citronelol, manoil oxide, myrcene, α -terpinene and p -cimene, significantly contribute to the grape brandy aroma and are specific only for distillates obtained from grapes (*Vitis vinifera* L.) Ledauphin et al. (2004).

Higher alcohols are mostly responsible for the pleasant fruity and floral aromas. Excepting terpenic alcohols assessed within the group of terpenic compounds, the majority of grape brandy samples were found to contain 6,10-dodecadiene-1-ol (samples II, V, VI and VII) and phytol (samples II, III, V, VI, VII). Their relative content was low, but their effect on grape brandy aroma, most notably that of phytol, was significant.

The analysis of the results on the number and relative content of acetals (Table 1) reveals that a total of 5 compounds belonging to this group were identified in the grape brandies from all cultivars. Given their low relative content, acetals

had a minor effect on the aroma of the grape brandies produced.

Among the aldehyde group, 3 compounds were identified in all grape brandy samples, the most abundant being hexadecanal and tetradecanal.

Eight components of the ketone class were identified in this study. The highest relative content of 10.24% was detected for 2-hydroxycyclopentadecanone in the brandy produced from cv. Early Muscat (sample II). The occurrence of other compounds was very significantly lower, ranging from 0.07% (t- β -damascenone) to 0.58% (3,3-diethoxy-2-butanone). The only exception was Dattier grape brandy (sample VIII) with no compound of this class being detected. Undoubtedly, the most important compound identified was t- β -damascenone, which was detected in the brandy produced from grape cv. Radmilovac Muscat. It is considered the key compound denoting an aroma factor in many alcoholic beverages, considering its very low sensory detection threshold in water (approximately 0.02 to 0.09 $\mu\text{g l}^{-1}$). Being responsible for the complex floral rose-like scent Genovese et al. (2004) and a cooked fruit-like aroma Ferrari et al. (2004), t- β -damascenone was found to affect the aromatic profile of the Radmilovac Muscat grape brandy (sample III). A high acetal concentration is often found in freshly distilled beverages. They are generally formed through mutual reaction of aldehydes with some alcohols (ethanol, butanol, etc.) Ledauphin et al. (2004). Ketones occur to a greater or lesser degree in almost all distilled beverages (Luiz Silva et al., 1996, Nikićević et al., 2000, Ledauphin et al., 2004). In view of the fact that some ketones have very low detection thresholds, they can contribute significantly to the aroma of distilled

beverages although they are present at low concentrations.

The aromatic hydrocarbons identified comprised compounds belonging to the alkane, alkane and alkenol groups, the most abundant of which were alkane compounds identified in distillates produced from all cultivars analysed.

There are no published data available on the effect of aromatic hydrocarbons on the aromatic profile of beverages distilled from grapes. Some of the above alkanes, such as cyclotetradecane and eicosane, have been identified in plum brandy Tesevic et al., (2005).

CONCLUSIONS

The results obtained on the relative content of volatile aromatic compounds in the grape brandies analysed suggest significant differences in both the number of aromatic components identified and their relative content. Given the unified grape brandy making technology, the

differences observed were induced solely by the cultivars used in grape brandy production.

The highest impact on the aroma of the grape brandies analysed was exhibited by terpenic compounds, followed by fatty acid esters.

Table 1.

Compounds identified in grape brandies produced from Muscat table grape (*Vitis vinifera* L.) cultivars, I-VIII (%)

Compound	I	II	III	IV	V	VI	VII	VIII
acetals								
2-propyl-1,3-dioxolane			0.23	0.8				
2-methoxy-2,3,3-trimethyl						0.26		
2,6-dimethyl-1,6-octadiene			0.39					
1,1-diethoxy-2-methyl propane							0.1	
1,1-diethoxy-3-methyl butane							0.05	
alcohols								
1-dodecanol		0.04						
1,5,7-octatrienol							0.08	
Benzyl alcohol	0.07					0.27		
1-tetradecanol							0.27	0.26
6,10-dodecadien-1-ol		0.09			0.11	0.13	0.06	
2,6,10-dodecatrien-1-ol					0.58			
Hexadecane-1,2-diol		0.05						
Phytol		0.06	0.18		0.13	0.08	0.06	
acids								
Octanoic acid		0.32	0.16	0.51	0.45	0.52		0.65
Decanoic acid	0.93	0.88	0.92	1.27			0.14	1.4
Dodecanoic acid	1.47	0.9	0.83	1.61	1.41	1.29	2.3	1.31
9,12-octadienoic acid	0.15			0.13	0.04			
Tetradecanoic acid	0.58		0.34	0.47	0.1	0.19	0.36	
9-hexadecenoic acid			0.53	0.11				

9-hexadecanoic acid	0.23					0.17		
Hexadecanoic acid		0.71				0.38	0.68	
9,12-octadecanoic acid		0.09						
7,10,13-hexadecadienoic acid		0.05						
9,12-octadecadienoic acid			0.09					0.05
10,13- octadecadienoic acid								0.08
9,15- octadecadienoic acid								0.09
9,12,15-octadecatrienoic acid								0.03
9-octadecenoic acid	0.73		0.2	0.61				
Nonadecanoic acid			0.05					
Linolenic acid	0.01		0.07	0.01				

Table 1. (Continued)

Compound	I	II	III	IV	V	VI	VII	VIII
esters								
Isoamyl acetate	0.13	1.31	0.72	0.76	0.82	1.45	0.33	
2-ethyl-3-hydroxy valerate		0.32						
Isopentyl acetate				0.16			0.07	
2-methylbutyl acetate			0.21		0.09	0.33		
1,1-diethoxy-3-methyl butane								
Ethyl hexanoate	1.32	0.68	0.74	1.1	1.86	1.84	1.7	0.95
1,1-diethoxy-hexanoate							0.05	
Methyl octanoate						0.05	0.05	
Linalyl acetate	0.28	0.79	0.8	0.12	0.08	0.79		
Ethyl benzoate							0.09	
Ethyl octanoate	1.79	3.61	3.4	4.47	13.98	14.64	15.0	21.56
Phenylethyl propanoate		0.23						
Amyl hexanoate							0.06	
3-methylbutyl octanoate					0.07	0.06		
2-fenilethyl acetate				0.18	0.13		0.05	
Phenylethyl propanoate						0.27		
Geranyl acetate	0.1	0.18	0.42	0.04	0.06	0.19	0.03	
Propyl octanoate							0.04	
Ethyl nonanoate	0.04	0.06			0.11	0.08		
Ethyl pelargonate							0.09	
Methyl decanoate				0.02	0.15	0.13	0.14	0.13
Isobutyl caprylate					0.07	0.06		
Isobutyl octanoate							0.06	
Citronelyl acetate	0.28	0.24	0.22	0.08	1.19	0.06	0.08	
Neryl acetate	0.16	0.12	0.11	0.06	0.32	0.05		
Ethyl-9-decanoate	0.33	0.32	0.24	0.2	2.4	6.69		1.18
Ethyl decanoate	3.29	7.44	4.55	7.06	25.46	22.06	25.12	30.57
Ethyl heptadecanoate							0.09	
Isoamyl octanoate	0.04	0.1	0.06	0.08	0.57	0.48	0.52	0.39
Isoamyl caprylate		0.03	0.07		0.13	0.1		0.08
3-methylbutyl octanoate							0.09	
Propyl decanoate			0.07		0.07	0.04	0.05	
Methyl dodecanoate		0.02			0.05	0.06	0.07	
Isobutyl decanoate		0.05		0.06	0.13	0.1	0.08	

Ethyl dodecanoate	4.39	7.17	4.65	6.9	14.19	11.38	11.71	12.56
Isoamyl butyrate	0.18		0.18	0.27	0.11	0.09		
3-methyl butyldecanoate					0.69		0.57	
Isoamyl decanoate	0.04	0.26	0.04	0.05	0.22	0.52	0.12	0.39
Isobutyl dodecanoate	0.05	0.04	0.05	0.05	0.05			
Ethyl tetradecanoate	2.52	1.91	2.53	2.39	1.83	1.61	2.77	1.91
Isoamyl dodecanoate	0.38	0.28		0.38		0.31	0.03	0.17
Isoamyl laurate			0.36					
Ethyl heptanoate			0.14		0.08	0.06		
2-phenylethyl octanoate	0.05	0.05		0.04	0.18	0.09	0.07	
Methyl-9,12-octadecanoate		0.07						
Methyl octadecanoate		0.05						
Ethyl 3-hydroxy tridecanoate		0.07						
Ethyl tridecanoate	0.27	0.16		0.23		0.05		

Table 1. (Continued)

Compound	I	II	III	IV	V	VI	VII	VIII
Citronellyl butirate	0.04							
Ethyl undecanoate			0.11				0.05	0.11
Methyl hexadecanoate	0.13	0.15	0.22	0.17	0.05	0.04	0.07	0.09
Ethyl 9-hexadecanoate	13.17	0.13	7.27	10.52	2.8	1.59	3.43	1.38
Ethyl hexadecanoate	17.75	18.1	17.06	17.88	6.91	5.81	8.2	6.41
2-phenylethyl octanoate			0.07	0.07	0.15			
Ethyl cyclooctadecane					0.08			
Ethyl heptadecanoate	0.14	0.09	0.14	0.09	0.08			
Methyl 9-octadecenoate	0.06						0.08	
Ethyl linolate					5.27		0.15	
Ethyl linoleate	18.97	10.62	15.52	15.18	4.18	4.63	0.19	4.94
Ethyl oleate		8.98	9.62			3.49	8.46	2.88
Ethyl 9,12,15-octadecatriene							4.71	
Ethyl 9-octadecenoate	11.48			10.1				
Ethyl stearate	1.51	1.69	1.93	1.93	0.81	0.62	0.71	0.4
terpenoids								
α -pinene	0.07		0.07	0.05		0.08	0.08	
β -myrcene	0.22	0.23		0.13		0.22	0.14	
α -terpinene	0.16	0.12	0.2	0.1		0.12	0.11	
<i>p</i> -cymene	0.2		0.16	0.14		0.33	0.14	
Limonene	7.53	7.55	8.7	5.84	1.34	8.35	6.17	
δ -3-carene			0.16					
<i>t</i> - β -ocimene	0.04		0.11					
γ -terpinene	1.6	1.29	1.72	1.25	0.22	1.62	1.22	
<i>c</i> -linalool oxide	0.06		0.11					
<i>t</i> -linalool oxide		0.19						
α -terpinolene	0.48	0.47	0.62	0.32	0.08	0.43	0.31	
Linalool	0.57	2.7	2.12	0.29	0.82	3.03	0.45	
Hotrienol	0.16	1.73	0.98	0.26	0.32	0.32		
<i>t</i> -rose oxide					0.04			
1,3,8-para-mentatriene		0.11	0.31					
Neroloxid	0.12	0.4	0.51	0.08	0.07	0.06		
α -terpienol	0.11	0.3	0.39	0.05				

Santene		0.06						
Citronellol	0.71	0.47	0.42		0.34	0.08	0.12	
Myrcenol			0.11					
2-carene		0.03						
β -pinene	0.06							
Geraniol	0.34	0.16	0.08			0.1	0.03	
Bornylene	0.16			0.05				
Vitispirane		0.06	0.06				0.05	
<i>trans</i> - β - caryophyllene		0.04	0.04					
Camphen			0.06					
<i>t</i> - β -farnesene		0.03	0.07					
α -bergamoten	0.06	0.02		0.04		0.05	0.04	
β -bisabolen		0.04	0.12		0.12	0.07		
Farnesol	0.31	0.19	0.13	0.13	0.44	0.26	0.12	
Fenchone					0.09		0.05	
β -fenchene	0.14	0.22	0.31	0.14				
Manoil oxide	0.26	0.08	0.41	0.25	0.12	0.06		
Farnesol	0.19			0.12				

Table 1. (Continued)

Compound	I	II	III	IV	V	VI	VII	VIII
aldehydes								
4-hydroxy-2-methoxy		0.32						
Hexadecanal	0.14	0.04	0.03	0.03				
Tetradecanal	0.07		0.09	0.1				
ketones								
3-nonanone		0.08						
2-cyclopenten-1-one	0.26			0.16				
<i>t</i> - β - damascenone			0.07					
2-heptadecanone	0.08							
Oxacyclotetradecan-2,11-dione			0.14		0.22			
2-pentyl-2-cyclopenten-1-one			0.14					
2-hydroxy cyclopentadecanone		10.24						
Farnesyl acetone			0.2		0.1			
Alkanes								
Cyclododecane	0.42	0.41	0.32	0.39		0.4		
Cyclodotetradecane					0.35			
Cyclotetradecane	1.21	0.06	0.05	1.23	0.51		0.29	
Cyclohexadecane						0.3		
9-eicosane			0.1					
Tricosane		0.17	0.12					
Octadecane			0.04					
Pentacosane		0.14						
Alkenes								
3-hexadecene							0.07	
1-hexadecene		1.22					0.13	
2-nonadecene	0.12			0.12				
1,13-tetradecadiene					0.15			
Alkenols								
8-nonene-2-ol	0.22							

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