Research Note

Protection of Volatiles in a Wine with Low Sulfur Dioxide by Caffeic Acid or Glutathione

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Abstract: Concentrations of aromatic volatiles during storage of white wine with reduced (35 mg/L) or typical (55 mg/L) free sulfur dioxide for up to 210 days (7 months) were measured to evaluate how decreased SO_2 affects wine volatiles. Additions of caffeic acid (60 mg/L), glutathione (20 mg/L), or their mixture (30 mg/L + 10 mg/L, respectively) to wine with reduced SO_2 were also examined. In control and treated wines, concentrations of acetate esters, ethyl esters, terpenes, and fatty acids decreased during wine storage, while concentrations of higher alcohols remained constant. Wine samples with reduced or typical SO_2 had statistically equal concentrations of volatiles, with the exception of ethyl acetate, which was higher in the latter. Caffeic acid, glutathione, or their mixture slowed the decrease of several volatile esters and terpenes such as ethyl acetate, isoamyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate, and linalool. Results suggest that SO_2 gives only limited protection to wine volatiles but that caffeic acid, glutathione, or their mixture protect several aromatic volatiles of white wine with reduced SO_2.

Key words: wine, volatiles, sulfur dioxide, phenolics, thiols

Oxidative spoilage of young white wines is a well-known problem in winemaking. The first step of oxidation is characterized by transformation of aromatic compounds, leading to loss of characteristic wine aromas and, subsequently, to formation of new aromas characteristic of older wines or atypical aromas associated with wine deterioration. Oxidative browning is a later step of wine oxidation (Ferreira et al. 1997, Ferreira et al. 2002, Singleton 1987).

Phenolic compounds participate in wine oxidation. Wines are rich in phenolic compounds that have an antioxidant effect on wine that acts as a natural preservative (Jackson 1994, Vaimakis and Roussis 1993). Inhibition of linalool degradation during oxidative storage of muscat wine by caffeic acid or gallic acid and inhibition of volatile ester and linalool degradation during oxidative storage of white and red wine by caffeic acid have been reported (Roussis et al. 2005a,b).

Amino acids and peptides containing sulfhydryl groups are good inhibitors of both enzymic and nonenzymic browning in a wide variety of foods. Among the most active is glutathione, a naturally occurring tripeptide (Friedman 1994). Among wine aroma compounds, glutathione and *n*-acetyl-cysteine reduced degradation of linalool during oxidative storage of muscat wine, and *n*-acetyl-cysteine inhibited degradation of volatile esters and linalool during oxidative storage of white and red wine (Papadopoulou and Roussis 2001, Roussis et al. 2005b).

Sulfur dioxide (SO_2) is the most common preservative used in winemaking. In addition to antimicrobial activity, it exhibits antioxidant properties and suppresses the activity of several oxidases and nonenzymatic oxidative reactions. However, at free SO_2 levels between 15 and 40 mg/L most individuals begin to detect a distinctive burnt match odor. Moreover, consumption of high concentrations of sulfite may have adverse health effects, such as asthma, on humans. As a result, the trend is to limit the use of SO_2 (Amerine and Roessler 1983, Jackson 1994). Here we determine the concentrations of aromatic volatiles during storage of a white wine containing reduced or typical free SO_2 and the effect on volatiles of adding caffeic acid, glutathione, or their mixture to wine containing reduced SO_2.

Materials and Methods

Caffeic acid and glutathione were purchased from Sigma (St. Louis, MO). The water used in the experiments was of HPLC grade (LabScan, Dublin, Ireland). Debina white wine was used (2004 vintage). Debina is a late ripening, easily oxidizable variety cultivated at Zitsa (Epirus, Greece), and Debina dry white wine is marked under this appellation of origin. The average composition of the white Debina wine was the following: alcohol content, 11.5% vol; pH, 3.45; residual sugar, <2 g/L); total acidity, 5.5 g/L as tartaric acid, and volatile acidity, 0.32 g/L as

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acetic acid. Reduced sulfur Debina wine had total SO_2 of 143 mg/L and free SO_2 of 35 mg/L, and the typical sulfur control had total SO_2 of 166 mg/L and free SO_2 of 55 mg/L. The antioxidant ascorbic acid (90 mg/L) was added to all wine samples, as it is common practice.

Gross composition of wine samples was determined by classic methods (Ough and Amerine 1988). Alcohol was determined with a hydrometer, reducing sugars by the Lane–Eynon method, pH with a pH meter, total acidity by volumetric analysis, and volatile acidity by steam distillation. Total and free SO_2 were determined by the Ripper method.

Wine samples (45 mL) containing 35 mg/L of free SO_2 were placed in 50-mL wine bottles and mixed with 0.2 mL of aqueous caffeic acid (final concentration, 60 mg/L), glutathione (20 mg/L), or a mixture of caffeic acid (30 mg/L) and glutathione (10 mg/L). Control samples were also prepared by adding 0.2 mL water to 45 mL wine containing 35 or 55 mg/L free SO_2, designated as reduced SO_2 (CR) and typical SO_2 (CT), respectively. The bottles were sealed using cork and sealing wax and stored in a dark room at 20°C. After 0, 90, and 210 days of storage, bottles were taken and wine samples were examined. (Debina white wine is easily oxidized and usually 50 to 55 mg/L free SO_2 is used. Consequently, we used wine samples containing typical [55 mg/L] or reduced [35 mg/L] free SO_2.)

All wine samples were analyzed for volatiles by solid-phase microextraction (SPME) gas chromatography-mass spectrometry (GC-MS). A 65-μm Carbowax/divinylbenzene fiber (Supelco, Bellefonte, PA) was used for the absorption of volatiles. Two mL of each wine or the model medium sample and 50 μL of internal standard in 10% ethanol (4-methyl-1-pentanol, 5 mg/L in final solution) was transferred into a 4-mL screwcap glass vial with a Teflon-rubber septum (12 mm; Sun-Sri, Rockwood, PA). The contents were stirred for 10 min at 35°C, and a constant length of fiber was then exposed to the headspace for another 15 min under the same conditions. Desorption of volatiles was at 240°C using a 0.75-mm i.d. liner (Supelco) for 10 min. Split/splitless mode was used for 2 min and split ratio was 1:20. GC-MS analysis was carried out on a HP 5973 quadrupole mass spectrometer directly coupled to a HP 6890 gas chromatograph (Hewlett-Packard, Palo Alto, CA). MS was operated in the electron impact mode with the electron energy set at 70 eV using G1701BA ChemStation. Source and quadrupole temperatures were set at 230 and 150°C, respectively. An Innowax fused-silica column was used (30 m x 0.32 mm, 0.5-μm film thickness) (J&W Scientific, Folsom, CA). The carrier gas was helium at a constant flow rate of 0.7 mL/min and average velocity of 30 cm/sec. The oven temperature was programmed from 40°C for 6.5 min, raised to 60, 220, and 250°C at rates of 2.0, 5.0, and 15°C/min, respectively, and then held at 250°C for 5.5 min. Mass range, 29 to 400 m/z, and 2.35 scan s^{-1} were applied, with solvent delay at 3.5 to 5.5 min to avoid the ethanol peak. The transfer line was kept at 260°C.

All peaks were identified by comparing mass spectra to those obtained from the Wiley 275 and NIST 98 libraries. Identification of many peaks was confirmed with mass spectra and comparison to retention times of standard compounds determined under the same conditions. Authentic standards used were ethyl acetate, isoamyl acetate, hexyl acetate, 2-phenyl ethyl acetate, ethyl lactate, ethyl caproate, diethyl succinate, ethyl caprylate, ethyl caprate, ethyl laurate, limonene (Merck, Darmstadt, Germany); linalool, α-terpineol, citronellol, isoamyl alcohol, benzene ethanol, hexanol, octanol, decanol (Aldrich, Milwaukee, WI); and caproic, caprylic, capric, and lauric acids (Sigma, St. Louis, MO). Semiquantitative data were expressed in milligrams per liter [(area of compound/area of internal standard) x concentration of internal standard].

Each experiment was repeated three times and results reported are means of the three trials. A one-way analysis of variance (ANOVA), using Duncan's test at $p < 0.05$, was used for statistical analysis (SPSS Inc., Chicago, IL).

Results

Concentrations of aromatic volatiles were determined in a white wine containing reduced (35 mg/L) or typical (55 mg/L) free SO_2 during storage for up to 210 days (Tables 1, 2, 3). The effect of adding caffeic acid (60 mg/L), glutathione (20 mg/L), or their mixture (30 mg/L + 10 mg/L, respectively) in wine containing reduced SO_2 on the levels of volatiles was examined. Concentrations of acetate esters, ethyl esters, terpenes, and fatty acids decreased during wine storage, while the concentrations of higher alcohols did not.

Wine samples containing reduced SO_2 showed statistically equal concentrations of volatiles to wines containing typical SO_2 at any sampling time (Table 2). The only exception was ethyl acetate, which was higher in wine with typical SO_2. No effect on the concentration of any volatile was observed at t = 0 as a result of adding caffeic acid, glutathione, or their mixture to wine with reduced SO_2. Caffeic acid, glutathione, or their mixture slowed the decrease of acetate esters, ethyl esters, and terpenes during wine storage, but had no effect on the total higher alcohols and fatty acids (Table 1). Many volatile esters and terpenes decreased during wine storage, including ethyl acetate, isoamyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate, and linalool. In contrast, ethyl lactate and diethyl succinate increased during wine storage. Caffeic acid, glutathione, or their mixture protected volatile esters and terpenes that decreased, but had no effect on the concentration of those that increased or were stable during wine storage (Table 2).

Discussion

Under the conditions of our experiments, volatile losses may be due to oxidation or other chemical reactions. For example, ester concentration may change because of hydrolysis and esterification (Ramey and Ough 1980).

Table 1 Sums of the total relative concentrations of volatile acetate esters, ethyl esters, terpenes, higher alcohols, and fatty acids of Debina white wine during storage at 20°C at 0, 90, and 210 days.

	CR[a] (mg/L)			CT (mg/L)			CR+C (mg/L)			CR+G (mg/L)			CR+C+G (mg/L)		
	0	90	210	0	90	210	0	90	210	0	90	210	0	90	210
Acetate esters	18.31Aa ± 1.86[b]	9.69 Ba ± 1.24	7.27Ca ± 0.54	17.67a ± 1.74	9.95a ± 1.15	8.54b ± 0.47	18.52a ± 1.79	14.72b ± 1.32	11.38d ± 0.62	18.14a ± 1.87	12.92b ± 1.08	9.91c ± 0.52	17.48a ± 1.69	15.02b ± 1.44	11.79d ± 0.66
Ethyl esters	271.58Aa ± 26.32	203.71Ba ± 16.74	125.14Ca ± 13.55	276.93a ± 28.56	207.71a ± 13.44	129.67a ± 12.88	277.30a ± 26.51	242.16b ± 16.63	184.07c ± 12.17	273.19a ± 27.46	234.39b ± 14.22	159.61b ± 13.29	280.65a ± 29.33	244.12b ± 16.38	188.13c ± 11.74
Terpenes	1.24Aa ± 0.23	0.79Ba ± 0.05	0.50Ca ± 0.08	1.11a ± 0.14	0.81a ± 0.04	0.53a ± 0.07	1.16a ± 0.17	0.92b ± 0.05	0.78c ± 0.05	1.16a ± 0.12	0.92b ± 0.05	0.68b ± 0.04	1.12a ± 0.18	0.97b ± 0.07	0.74bc ± 0.07
Higher alcohols	82.33Aa ± 3.45	81.78Aa ± 4.66	78.54Aa ± 3.72	81.79a ± 4.61	81.21a ± 2.85	77.27a ± 3.45	80.56a ± 3.12	79.85a ± 2.63	78.17a ± 2.77	82.08a ± 2.67	80.56a ± 3.02	79.45a ± 3.55	81.66a ± 2.64	80.67a ± 2.79	78.24a ± 2.99
Fatty acids	27.95Aa ± 2.55	24.12Aa ± 2.78	17.05Ba ± 3.88	28.13a ± 3.79	22.69a ± 4.88	15.77a ± 3.41	26.55a ± 3.21	23.18a ± 2.93	14.88a ± 3.13	27.44a ± 3.17	23.78a ± 3.82	16.85a ± 3.43	28.71a ± 2.87	23.66a ± 3.56	16.73a ± 3.74

[a]Abbreviations, CR: control with reduced free SO_2 (35 mg/L); CT: control with typical free SO_2 (55 mg/L); CR+C: CR + caffeic acid (60 mg/L); CR+G: CR + glutathione (20 mg/L); CR+C+G: CR + caffeic acid (30 mg/L) + glutathione (10 mg/L).

[b]Values, mg/L as 4-methyl-1-pentanol, are the means of three trials. Different capital letters indicate significant differences among CR samples stored for 0, 90, and 210 days.

Moreover, ester oxidation by hydroxyl-radical oxidation related processes has also been proposed by some authors (Litchev 1989, Escudero et al. 2000). Geraniol and nerol can interconvert and then form α-terpineol (Pedersen et al. 2003) and linalool may be replaced by α-terpineol (Jackson 1994).

All acetates and many ethyl esters decreased during storage of Debina wine, while ethyl lactate and diethyl succinate did not. These results are similar to those observed during oxidative storage of other wines (Ferreira et al. 1997, Roussis et al. 2005). Linalool and α-terpineol also decreased during storage of Debina wine. Similar decreases of these two terpenes during oxidative storage of muscat wine has been reported (Roussis et al. 2005a).

Volatile higher alcohols were stable during storage of Debina wine. Similar stability during oxidative storage of other wines has been reported (Ferreira et al. 1997, Roussis et al. 2005b). Most fatty acids, including caproic, caprylic, and capric, decreased during storage of Debina wine. There are conflicting reports on fatty acid concentration during oxidative storage of other wines (Ferreira et al. 1997, Roussis et al. 2005b).

In the present study, wine samples with reduced or typical free SO_2 exhibited similar volatile concentrations. Results indicate that SO_2 may not play a crucial role in protection of volatiles during wine storage. Moreover, addition of caffeic acid, glutathione, or their mixture protected several volatile esters and terpenes during storage of wine containing less free SO_2 than typical. Among them were several important to wine aroma, such as isoamyl acetate, ethyl caproate, ethyl caprylate, ethyl caprate, and linalool (Jackson 1994). The inhibitory action of caffeic acid and glutathione may be related to their antioxidant properties. However, the mechanism that causes esters to disappear during wine storage is unknown.

Caffeic acid and glutathione are natural constituents of wine. Caffeic acid is the main hydroxycinnamic acid in grapes and wine, and its ester with tartaric acid (caftaric acid) is predominant in grapes, averaging 170 mg/kg. In the aqueous acidic solution of wine, hydroxycinnamates are susceptible to hydrolysis, releasing simple hydroxycinnamic acids, which are then partially esterified with ethanol. Concentrations of total hydroxycinnamates in wine are typically 130 mg/L in whites and 60 mg/L in reds (Waterhouse 2002). Glutathione increases at the onset of grape berry ripening and during fermentation. Its concentration in wine may be 2 to 5 mg/L (Adams and Liyanage 1993, Park et al. 2000). It is possible that control of caffeic acid and glutathione concentrations in wines is critical in making high-quality white wines.

Conclusion

Present results indicate that addition of caffeic acid, glutathione, or their mixture may permit reduced SO_2 addition to white wines while still protecting several aromatic volatiles.

Table 2 Concentrations of volatile acetate esters, ethyl esters, and terpenes of Debina white wine during storage at 20°C at 0, 90, and 210 days of storage.

	0 days	90 days					210 days				
Volatiles	**CR[a]**	**CR**	**CT**	**CR+C**	**CR+G**	**CR+C+G**	**CR**	**CT**	**CR+C**	**CR+G**	**CR+C+G**
Ethyl acetate	8.63A[b] ± 1.26	4.66Ba ± 0.90	4.73[a] ± 0.48	6.84b ± 0.72	6.11b ± 0.54	6.95b ± 0.68	3.77Ba ± 0.37	4.62b ± 0.32	5.92d ± 0.38	5.22c ± 0.27	6.07d ± 0.42
Isoamyl acetate	6.73A ± 1.18	3.55Ba ± 0.41	3.64a ± 0.34	5.55c ± 0.47	4.87b ± 0.27	5.76c ± 0.37	2.51Ca ± 0.34	2.87a ± 0.30	4.05c ± 0.27	3.54b ± 0.26	4.28c ± 0.32
Hexyl acetate	1.05A ± 0.18	0.46Ba ± 0.09	0.51Ba ± 0.10	0.75b ± 0.07	0.59a ± 0.05	0.72b ± 0.06	0.35Ba ± 0.03	0.38a ± 0.04	0.47b ± 0.03	0.42ab ± 0.04	0.45b ± 0.04
Ethyl phenyl acetate	0.08A ± 0.02	0.03Ba ± 0.01	0.03Ba ± 0.01	0.05a ± 0.02	0.03a ± 0.01	0.03a ± 0.01	0.02Ba ± 0.01	0.02a ± 0.01	0.05a ± 0.03	0.02a ± 0.02	0.05a ± 0.03
2-Phenyl ethyl acetate	1.82A ± 0.10	0.99Ba ± 0.11	1.04a ± 0.09	1.53b ± 0.14	1.32b ± 0.10	1.56b ± 0.13	0.62Ca ± 0.07	0.65a ± 0.07	0.89b ± 0.08	0.71a ± 0.06	0.94b ± 0.05
Ethyl isovalerate	0.10A ± 0.05	0.04Aa ± 0.02	0.05a ± 0.03	0.07a ± 0.04	0.05a ± 0.02	0.08a ± 0.03	0.00Ba ± 0.00	0.00a ± 0.00	0.02ab ± 0.01	0.00a ± 0.00	0.03b ± 0.01
Ethyl caproate	20.68A ± 3.02	13.74Ba ± 1.87	13.94a ± 1.45	16.99b ± 1.66	16.21b ± 1.37	17.31b ± 1.53	9.02Ca ± 1.15	9.89a ± 1.24	14.23b ± 1.58	12.23b ± 1.46	11.89b ± 1.27
Ethyl lactate	4.25A ± 0.75	4.95Aa ± 0.31	4.88a ± 0.22	4.25a ± 0.38	4.45a ± 0.34	4.57a ± 0.27	5.24Ba ± 0.57	5.14a ± 0.62	4.59a ± 0.51	4.78a ± 0.44	5.01a ± 0.49
Ethyl caprylate	127.16A ± 7.24	103.52Ba ± 4.72	106.13a ± 5.11	117.67b ± 4.63	114.31ab ± 4.78	118.25b ± 5.08	63.21Ca ± 3.77	66.54a ± 4.55	92.08c ± 4.76	78.89b ± 4.18	95.12c ± 4.38
Ethyl pelargonate	0.41A ± 0.07	0.18Ba ± 0.04	0.21a ± 0.03	0.34b ± 0.04	0.28b ± 0.03	0.31b ± 0.03	0.10Ba ± 0.04	0.11a ± 0.04	0.26b ± 0.04	0.16a ± 0.03	0.21b ± 0.02
Ethyl caprate	103.12A ± 10.24	68.85Ba ± 5.34	70.12a ± 4.87	90.22b ± 5.67	86.54b ± 4.92	90.78b ± 5.12	37.54Ca ± 4.79	38.05a ± 4.66	62.1b ± 5.21	53.14b ± 6.64	65.20b ± 5.68
Diethyl succinate	4.97A ± 0.52	6.68Bb ± 0.63	6.60b ± 0.52	5.34a ± 0.53	5.89ab ± 0.45	5.78ab ± 0.47	7.25Ba ± 1.12	7.01a ± 1.14	6.52a ± 1.23	7.12a ± 0.88	6.95a ± 0.94
Ethyl-9-decanoate	0.43A ± 0.12	0.28Ba ± 0.06	0.29a ± 0.05	0.36a ± 0.06	0.30a ± 0.07	0.37a ± 0.06	0.12Ca ± 0.04	0.14a ± 0.03	0.23b ± 0.04	0.17ab ± 0.03	0.19ab ± 0.03
Ethyl laurate	6.98A ± 0.91	3.12Ba ± 0.31	3.10a ± 0.27	4.02b ± 0.25	3.74b ± 0.24	3.92b ± 0.21	1.15Ca ± 0.18	1.23a ± 0.22	2.31b ± 0.24	1.54a ± 0.17	2.04b ± 0.28
Ethyl-3-methylbutyl-butanedioate	0.35A ± 0.07	0.39Aa ± 0.02	0.40a ± 0.03	0.38a ± 0.02	0.39a ± 0.03	0.37a ± 0.02	0.40Aa ± 0.03	0.41a ± 0.03	0.42a ± 0.02	0.40a ± 0.03	0.41a ± 0.02
Ethyl myristate	2.07A ± 0.55	1.35Aa ± 0.17	1.36a ± 0.16	1.71b ± 0.15	1.54ab ± 0.18	1.62ab ± 0.20	0.87Ba ±0.08	0.89a ± 0.06	0.94a ± 0.07	0.90a ± 0.05	0.92a ± 0.05
Ethyl palmitate	1.06A ± 0.24	0.61ABa ± 0.10	0.63a ± 0.07	0.81b ± 0.06	0.69ab ± 0.08	0.76ab ± 0.06	0.24Ba ± 0.05	0.26a ± 0.04	0.37b ± 0.05	0.28ab ± 0.06	0.33ab ± 0.05
Limonene	0.10A ± 0.02	0.00Ba ± 0.00	0.00a ± 0.00	0.00a ± 0.00	0.00a ± 0.00	0.00a ± 0.00	0.00Ba ± 0.00	0.00a ± 0.00	0.00a ± 0.00	0.00a ± 0.00	0.00a ± 0.00
Linalool	0.87A ± 0.12	0.61Ba ± 0.04	0.63a ± 0.04	0.72b ± 0.03	0.68ab ± 0.04	0.75b ± 0.05	0.39Ca ± 0.03	0.41a ± 0.04	0.59bc ± 0.03	0.52b ± 0.03	0.65c ± 0.04
α-Terpineol	0.16A ± 0.03	0.10Aa ± 0.02	0.12a ± 0.02	0.11a ± 0.02	0.13a ± 0.02	0.12a ± 0.03	0.05Ba ± 0.02	0.06a ± 0.01	0.10b ± 0.02	0.08ab ± 0.02	0.011b ± 0.03
Citronellol	0.11A ± 0.03	0.08ABa ± 0.02	0.07a ± 0.02	0.09a ± 0.02	0.11a ± 0.03	0.10a ± 0.02	0.06Ba ± 0.02	0.06a ± 0.01	0.09a ± 0.02	0.08a ± 0.01	0.08a ± 0.02

[a]Abbreviations. CR: control containing reduced free SO_2 (35 mg/L); CT: control containing typical free SO_2 (55 mg/L); CR+C: CR + caffeic acid (60 mg/L); CR+G: CR + glutathione (20 mg/L); CR+C+G: CR + caffeic acid (30 mg/L) + glutathione (10 mg/L).

[b]Values, mg/L as 4-methyl-1-pentanol, are the means of three trials. Different capital letters indicate significant differences among CR samples stored for 0, 90, and 210 days. Different lowercase letters indicate significant differences among all treatments after the same length of storage.

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Table 3 Concentrations of volatile higher alcohols and fatty acids of Debina white wine during storage at 20°C and at 0, 90, and 210 days.

	0 days	90 days	210 days
Propanol	1.31A ± 0.10[a]	1.19A ± 0.08	0.84B ± 0.10
2-Methyl-propanol	3.43A ± 0.41	2.99AB ± 0.34	2.57B ± 0.32
Isoamyl alcohol	55.41A ± 2.14	56.11A ± 2.45	54.18A ± 2.77
Hexanol	1.97A ± 0.09	1.86A ± 0.07	1.62B ± 0.08
cis-3-Hexenol	0.40A ± 0.06	0.32A ± 0.03	0.25B ± 0.03
2-Ethyl-hexanol	0.24A ± 0.05	0.30A ± 0.06	0.27A ± 0.05
1,3-Butanediol	0.18A ± 0.09	0.15A ± 0.07	0.15A ± 0.08
Octanol	0.38A ± 0.13	0.36A ± 0.14	0.29A ± 0.12
2,3-Butanediol	0.51A ± 0.15	0.49A ± 0.16	0.45A ± 0.14
Methionol	0.21A ± 0.04	0.18A ± 0.04	0.15A ± 0.05
Decanol	0.13A ± 0.04	0.08A ± 0.04	0.07A ± 0.04
Benzene methanol	0.39A ± 0.05	0.34A ± 0.03	0.27B ± 0.02
Benzene ethanol	17.14A ± 0.44	16.77A ± 0.58	16.11A ± 0.63
Dodecanol	0.09A ± 0.02	0.07A ± 0.01	0.05B ± 0.01
Butanoic acid	0.31A ± 0.05	0.25AB ± 0.04	0.18B ± 0.05
3-Methyl-butanoic acid	0.38A ± 0.04	0.25B ± 0.04	0.14C ± 0.03
Caproic acid	3.14A ± 0.24	2.30B ± 0.21	1.67C ± 0.18
Caprylic acid	8.47A ± 0.31	6.12B ± 0.24	4.49C ± 0.19
Capric acid	8.82A ± 0.54	6.53B ± 0.42	3.48C ± 0.46
Lauric acid	0.53A ± 0.12	0.44A ± 0.06	0.33B ± 0.04
Myristic acid	3.47A ± 0.27	3.17A ± 0.31	2.16B ± 0.24
Palmitic acid	3.01A ± 0.38	3.63A ± 0.42	3.32A ± 0.55

[a]Values, mg/L as 4-methyl-1-pentanol, are the means of three trials. Different capital letters indicate significant differences in concentration after 0, 90, and 210 days of storage.

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