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Full Length Research Paper

Changes in aromatic compounds of cabernet sauvignon wines during ageing in stainless steel tanks

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The influence of age on the volatile composition of cabernet sauvignon red wines, aged in stainless steel tanks during different years was studied. For this purpose, the evolution of volatile compounds: alcohols, esters, fatty acids, aldehydes and ketones, of the four wines were determined using headspace solid phase microextraction (HS-SPME) and gas chromatography mass spectrometry (GC-MS). Quantitatively, alcohols formed the most abundant group in the aromatic components of the four wines studied, followed by esters and acids. The sum of the individual aroma compounds studied increased progressively, and the compounds that changed significantly were alcohols and esters. The profiles of all the aroma compounds for cabernet sauvignon wines were increasingly diverse. The ability to distinguish the aroma of the four wines was probably due to the dominance of alcohols, ethyl esters of fatty acids, and their contributions to the global aroma.

Key words: Cabernet sauvignon, stainless steel tanks, aromatic compounds, headspace/solid-phase microextraction, gas chromatography-mass spectrometry (GC/MS).

INTRODUCTION

Aroma compounds play an important role in the quality of wine because these compounds produce a sensory effect on the sense (Rapp, 1990). The aroma of wine depends on the balance of several hundred volatile compounds, whose individual concentrations vary between 10^{-1} and 10^{-10} g/L (Rapp and Mandery, 1986). These compounds have different origins; from grapes (varietal aroma), alcoholic fermentation under anaerobic conditions (fermentative aroma), and from the bouquet, which results from the transformation of the aroma during aging (Câmara et al., 2006a). The main groups of compounds that forms the fermentation bouquet are esters, alcohols, acids, and, to a lesser extent, aldehydes (Lambrechts and Pretorius, 2000). The bouquet is formed mainly by volatile esters, aldehydes, volatile aroma compounds (Li et al., 2005).

The process of aging wine is a fundamental step toward obtaining a high quality wine. During this period, the wine matures, and several processes take place that improve its sensory characteristics. In particular, the wine acquires aromatic complexity as a result of important modifications derived from esterification, hydrolysis, redox reactions, spontaneous clarification, CO_2 elimination, and slow and continuous diffusion of oxygen (Câmara et al., 2006b).

Alcoholic fermentation can be carried out in different types of containers, including stainless steel tanks, plastic tanks, and oak barrels. The use of oak barrels for fermenting wine might have a significant influence on the aromatic composition of the product. Wood is a porous material that can bind and release compounds, unlike the stainless steel tank, which is made of a material that does not interact with wine (Marco et al., 2008). Alcoholic fermentation of white must is usually carried out in stainless steel tanks after juice clarification. This type of container allows the winemaker to control the fermentation temperature and thus produce crisp white wines without any complication. However, it is worth noting that not all wines are suitable for aging in oak barrels because the oxygen could oxidize the wine, and the wood-derived components could completely gloss over its sensory characteristics (Liberatore et al., 2010).

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The results obtained showed that wine fermented in barrels had a greater concentration of higher alcohols and esters than those fermented in tanks (Liberatore et al., 2010). The concentrations of isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate, were four times higher in wine fermented in oak barrels compared to those fermented in stainless steel tanks (Liberatore et al., 2010). With regard to the concentration of acids, a greater concentration of medium-chain fatty acids (C6:0–C10:0) was noticeable in wine fermented in oak barrels. Given that these acids are toxic for the yeasts; this may be responsible for the slower fermentation rate of wine fermented in oak barrels (Marco et al., 2008).

With wine fermentation and aging in large stainless steel tanks becoming increasingly common for industrialized and going-to-scale production, it is necessary to elucidate the variation trend of the aroma derived from wine stored in stainless steel tanks. The aim of this work was to study the aromatic compounds of cabernet sauvignon wines stored in stainless steel tanks, as well as to analyze the sensory descriptors during aging.

MATERIALS AND METHODS

Winemaking

Test samples of cabernet sauvignon (*Vitis vinifera* L.c v. Cabernet Sauvignon) single variety grapes were harvested from Manasi, Sinkiang Province, China, on the 3rd of September, 2006, and they were vinificated at the Suntime Wine Company. The wine was fermented in 30 T stainless steel tanks with activated dry yeast (LAFFORT Company, France) and the traditional vinification process (Zhang et al., 2007). In late October, the wines were transferred to 120 T stainless steel tanks for storage and aging after sulfur dioxide, pumping over, racking (lees and wine), clarification, and malolactic fermentation in sequence. During the storage period, the liquor containers appeared to have head space due to the influence of pumping over, gases volatilizing, wine evaporation, and other natural conditions; the head space must be filled in a timely manner with wines of the same variety and age, and it cannot be filled with nitrogen to insulate oxygen. In general, pumping over was carried out 1 to 2 times monthly, or once a week in special circumstances. To maintain health management, the wines needed visual inspection every month, sensory checks each quarter, and detection of physiochemical indexes, especially the volatile acids. Starting in 2006, samples were collected every 12 months, in November 2006, 2007, 2008 and 2009.

Preparation of samples

Aromatic compounds of the wine samples were extracted by solid-phase microextraction and analyzed using gas chromatography/mass spectrometry, as described by Zhang et al. (2007). Five ml of wine and 1 g NaCl were placed in a 15 ml sample vial. The vial was tightly capped with a PTFE-silicon septum and heated at 40℃ for 30 min on a heating platform with agitation at 400 rpm. The Headspace Solid-phase Microextraction (HS-SPME) (50/30 µm DVB/CAR/PDMS, Supelco, Bellefonte, Pa., USA), preconditioned according to the manufacturer's instructions, was then inserted into the headspace, where extraction was allowed to occur for 30 min with continued heating and agitation by a magnetic stirrer. The fiber was subsequently desorbed in the Gas Chromatography (GC) injector for 25 min.

Gas chromatography–mass spectrometry (GC-MS) analysis

The GC-MS system used was an Agilent 6890 GC equipped with an Agilent 5975 mass spectrometer. The column used was a 60 m × 0.25 mm HP-INNOWAX capillary with 0.25 µm film thickness (J&W Scientific, Folsom, Calif., USA). The carrier gas was helium at a flow rate of 1 ml/min. Samples were injected by placing the SPME fiber at the GC inlet for 25 min with the splitless mode. The oven's starting temperature was 50℃, which was held for 1 min then raised to 220℃ at a rate of 3℃/min and held at 220℃ for 5 min. The mass spectrometry in the electron impact mode (MS/EI) at 70 eV was recorded in the m/z range of 20 to 450 U. The mass spectrophotometer was operated in the selective ion mode under auto-tune conditions, and the area of each peak was determined by ChemStation software (Agilent Technologies) (Zhang et al., 2007).

The mass spectrometric datum of each component was automatically searched in the NIST05 standard library; this was followed by checking and confirming the computer retrieval results relative to a reference standard spectrogram; then, according to the standard samples, we made a standard curve for calculating each group's concentrations.

Statistical analysis

One-way ANOVA was used to evaluate differences in the aromatic composition resulting from different aging periods of the wines. A significant difference was calculated at 0.05 levels. DPS version 7.55 Statistical Package for Windows was used for all statistical analysis.

RESULTS AND DISCUSSION

Main kinds of volatile compounds in the four wines

The key aromatic compounds of the wines were identified and grouped into alcohols, esters, acids, aldehydes, and ketones, as listed in Table 1.

Alcohols

Among the tested parameters, alcoholic degree was the enological parameter that had the greatest effect on the accumulation of volatile compounds in the wines (Garde et al., 2008). Quantitatively, alcohols formed the most abundant group in the aromatic components of the four wines, constituting 44.668 to 85.836% (relative value) of the total aroma content; followed by esters (6.221 to 12.355%, relative value) and acids (0.489 to 1.005%, relative value). This result was different from those in which acids formed the most abundant group reported by Zhang et al. (2007). In Zhang et al.'s (2007) research, ethanol was not considered in spite of its highest content in all the wines. Alcohols with 31 compounds represented the largest group in terms of the numbers of aromatic

Table 1. The aromatic compounds found in different vintages and their aroma descriptions.

Number of kind	Aroma component	Aroma description	Content of aroma component							
			06-11 (year-month)		07-11 (year-month)		08-11 (year-month)		09-11 (year-month)	
			Concentration (µg/L)	Relative content (%)	Concentration (µg/L)	Relative content (%)	Concentration (µg/L)	Relative content (%)	Concentration (µg/L)	Relative content (%)
Alcohol										
1	Ethyl alcohol	Alcoholic odor	nqw	5.194	nqw	18.2345	nqw	17.844	nqw	14.684
2	1-Propanol	Bouquet, ripe fruity odor	3058.80b	0.334	2554.87[d]	0.627	2682.83[c]	0.5	5091.44[a]	0.63
3	2-Methyl-1-propanol	Bitter apricot seed odor	nqw	2.2925	nqw	1.124	nqw	1.26	nqw	1.403
4	1-Butanol	Intoxicated aroma, alcoholic odor	3617.84[a]	0.163	3166.73[b]	0.058	2561.62[d]	0.045	2929.83[c]	0.053
5	2-Hexanol, (R)-	Coconut odor	nqw	0.141	-	-	-	-	-	-
6	2-Octanol	Unpleasant aromatic plant odor	-	-	-	-	-	-	nqw	0.056
7	4-Methyl-2-pentanol	-	nqw	0.051	nqw	0.054	nqw	0.08	nqw	0.064
8	3-Methyl-1-butanol	Cheese odor	nqw	71.875	nqw	23.015	nqw	24.8	nqw	28.205
9	1-Pentanol	Bouquet, astringent	-	-	-	-	nqw	0.00	nqw	0.01
10	4-Methyl-1-pentanol	-	7724.32[c]	0.013	12143.23[a]	0.024	7114.04[d]	0.013	9623.65[b]	0.018
11	2-Heptanol	Brass odor, lemon odor	-	-	-	-	nqw	-	nqw	-
12	3-Methyl-1-pentanol, (S)-(+)-	-	nqw	0.052	-	-	nqw	0.032	nqw	0.046
13	1-Hexanol	Light branches, leaves and fruity odor	4468.30[c]	1.354	4252.04[d]	0.57	8940.44[a]	0.9	6797.87[b]	0.784
14	3-Hexen-1-ol, (E)-	Strong fruity odor, light leafiness and green grass odor	-	-	-	-	909.76[a]	0.018	643.07[b]	0.012
15	3-Ethoxy-1-propanol	-	-	-	-	-	-	-	nqw	0.031
16	3-Hexen-1-ol, (Z)-	Strong fruity odor, light leafiness and green grass odor	-	-	-	-	889.05[a]	0.021	625.08[b]	0.013
17	2-Hexen-1-ol, (Z)-	-	-	-	-	-	-	-	nqw	0.00
18	2-Hexen-1-ol, (E)-	-	-	-	-	-	-	-	158.21[a]	0.013
19	1-Octen-3-ol	-	-	-	-	-	nqw	-	nqw	-
20	1-Heptanol	Bouquet plant odor, grape odor	nqw	0.01	-	-	-	-	-	-
21	2-Ethyl-1-hexanol	-	-	-	-	-	1667.97[a]	0.017	nq	-
22	3-Ethyl-4-methylpentanol, (S)-	-	nqw	0.105	nqw	0.014	nqw	0.039	nqw	0.047
23	2-Nonanol	Strong fruity odor, rose odor	-	-	-	-	21031.05[b]	0.008	41059.21[a]	0.037
24	2,3-Butanediol, [R-(R*,R*)]-	-	nq	0.58	nq	0.048	140.02[b]	0.062	361.08[a]	0.148

Table 1.cont.

25	2,3-Butanediol	Like rubber chemical odor	59.825[c]	0.00	394.20[a]	0.205	nq	0.1	220.79[b]	0.122
26	1-Octanol	Fresh oranges and rose odor	37210.27[a]	0.182	12311.96[d]	0.03	18828.28[c]	0.045	21787.41[b]	0.046
27	1-Nonanol	-	nqw	0.108	nqw	0.015	nqw	0.03	nqw	0.032
28	2-Furanmethanol	-	-	-	-	-	nqw	-	nqw	-
29	3-(methylthio)-1-Propanol	Raw potatoes odor, alliaceous odor	-	-	-	-	-	-	111.80[a]	0.015
30	Benzyl Alcohol	Bitter apricot seed odor	-	-	-	-	198.00[b]	0.00	292.57[a]	0.019
31	Phenylethyl Alcohol	sweet rose odor	nq	3.381	nq	0.65	nq	0.651	6477.77	1.169
Subtotal (%)			-	85.836	-	44.668	-	46.465	-	52.231
Ester										
32	Ethyl Acetate	Fruity odor, ester odor	8094.22[a]	1.712	7878.77b	2.1	7983.52[ab]	2.733	4684.98[c]	1.744
33	Ethyl butyrate, 3-methyl-	Fruity odor, fennel odor	-	-	-	-	nqw	0.036	nqw	0.044
34	Ethyl hexanoate	Green apple odor, fruity odor	nqw	2.38	nqw	0.7	nqw	0.827	nqw	0.77
35	Pentyl acetate, 1-Ethyl-	-	-	-	-	-	-	-	nqw	-
36	Hexyl acetate	Pleasant fruity odor, pear odor	-	-	nqw	0.015	nqw	0.8	-	-
37	Ethyl propionate, 2-hydroxy- , (S)-	-	nqw	0.127	nqw	0.532	nqw	1.1	nqw	1.33
38	Ethyl octanoate	Fruity odor, fennel odor, sweet odor	nqw	5.98	nqw	1.554	nqw	1.35	nqw	1.92
39	Methyl octanoate	Strong orange odor	214082.2[a]	0.898	-	-	nq	-	2735.57[b]	0.014
40	Isopentyl hexanoate	Fruity odor, fresh banana odor	-	-	-	-	-	-	nq	-
41	Ethyl butyrate, 3-hydroxy-	Fruity odor, strawberry odor	-	-	-	-	-	-	nq	-
42	Isoamyl lactate	-	-	-	nqw	0.039	nqw	0.1	nqw	0.145
43	Ethyl decanoate	Fruity odor	nqw	1.07	nqw	0.121	nqw	0.107	nqw	0. 12
44	Butyrolactone	-	-	-	-	-	nq	-	nq	-
45	Diethyl succinate		nq	0.188	nq	1.16	nq	1.302	12187.60[a]	1.385
46	Ethyl 9-decenoate	-	-	-	-	-	-	-	nq	-
47	2-phenylethyl propionate	-	-	-	-	-	nq	0.014	-	-
Subtotal (%)			-	12.355	-	6.221	-	8.369	-	7.472
Acid										
48	Acetic acid	Strong smell	18634.99[c]	0.484	31673.81[a]	0.345	nq	0.327	24020.78[b]	0.239
49	2-hydroxy-4-methyl-Pentanoic acid, (.+/-.)-	-	-	-	-	-	nq	-	nq	0.044
50	2-methyl-Propanoic acid	-	nq	0.1	-	-	-	-	-	-

Table 1 cont.

51	Butanoic acid	Unpleasant pickled odor, cheese odor	-	-	-	-	nq	-	nq	-
52	Hexanoic acid	Unpleasant copra oil odor	435.27[c]	0.136	419.45[c]	0.044	589.81[a]	0.078	523.95[b]	0.501
53	Octanoic Acid	Light fruity acid odor	516.42[d]	0.285	572.96[c]	0.1	784.33[b]	0.109	859.17[a]	0.108
54	Dodecanoic acid	Nut odor, metal odor	-	-	-	-	-	-	nq	-
55	n-Decanoic acid	-	-	-	-	-	-	-	nq	-
Subtotal (%)			-	1.005	-	0.489	-	0.514	-	0.892
Aldehyde and ketone										
56	Nonanal	Rose odor	-	-	-	-	-	-	nq	-
57	Furfural	Toast, fruity, floral odor	-	-	-	-	-	-	1907.18[a]	0.064
58	Benzaldehyde	-	-	-	128.03[b]	0.015	-	-	401.39[a]	0.058
59	5, 2--methyl-Furan carboxaldehyde	-	-	-	-	-	-	-	nq	-
60	Acetoin	Cream odor	-	-	-	-	nq	-	-	-
Subtotal (%)			-	-	-	0.015	-	-	-	0.122
Others										
61	3-Furaldehyde	-	-	-	-	-	nqw	0.001	-	-
62	Oxime-, methoxy-phenyl-	-	nqw	0.642	nqw	0.395	nqw	0.349	nqw	0.387

The data are mean values of triplicate samples; the different letters are significantly different ($P < 0.05$). nqw: Not quantified (without standards). nq: Not quantified (detection limit < concentration < quantification limit).

compounds identified. The most abundant alcohol found was 3-methyl-1-butanol, which produces the intoxicating fragrance of fresh wines (Li, 2006); it constituted 71.875, 23.015, 24.8, and 28.205% (relative value) of the total aroma content of the four wines. It was however, significantly higher in the 2006 wine. The alcohol profile of the 2009 wine was more diverse, containing 29 types of alcohols compared to only 17, 14, and 24 in the 2006, 2007, and 2008 wines, respectively. In a way, this phenomenon may explain why the flavor of some wines continues to become increasingly complex during aging process. 2-octanol, 3-ethoxy-1-propanol, (Z)-2-hexen-1-ol, (E)-2-hexen-1-ol, 4-trimethyl-3-cyclohexene-1-methanol, and 3-(methylthio)-1-propanol were only present in the 2009 wine. The content of most of the alcohols diminished with time, but more alcoholic compounds corresponded to a higher quality wine, as this contributed to the wine becoming increasingly complex.

Esters

There were also significant differences in the type and amount of esters present in the four wines. In general, the numbers of esters in the 2008 wine (12) and 2009 wine (14) were higher than those of the 2006 wine (7) and 2007 wine (8). Although, their amounts varied among the four wines, ethyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were the major esters found in the aromatic components of the four wines. 1-ethyl-pentyl acetate, isopentyl hexanoate, 3-hydroxy-ethyl butyrate, and ethyl 9-decenoate were esters found only in the 2009 wine, while 2-phenylethyl propionate was unique to the 2008 wine. Most neutral esters in wine (for example ethyl acetate and ethyl lactate) are biochemical esters produced mainly by yeast and bacterial activity. Then, in the aging process, the wine

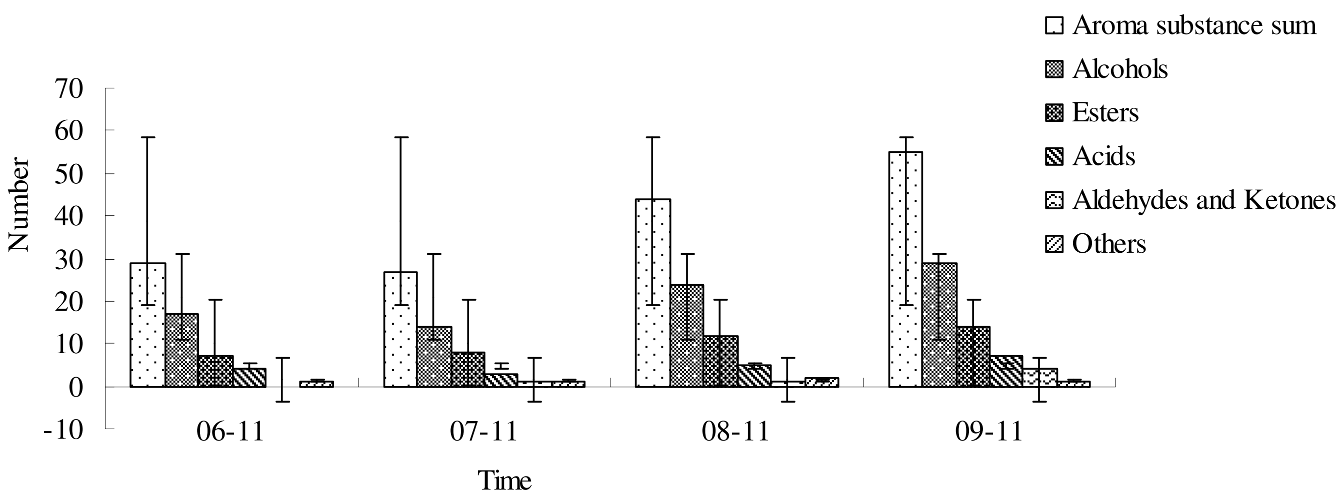

Figure 1. Variation of aromatic compounds in the four wines.

mainly produces acid ester (ethyl tartrate, ethyl succinate, etc.), and the esterification is very slow (Li et al., 2005). The ethyl esters of the medium-chain fatty acids (C_6–C_{12}) are produced during yeast fermentation by the reactions of ethanol and acyl-coenzyme A derivatives (Nordest et al., 1975). These compounds appear mainly during the alcoholic fermentation phase (Gil et al., 2006). On the other hand, the formation of acetic esters is the result of the reaction between acetyl-CoA and alcohols (Lee et al., 2004).

Acids

The production of fatty acids has been reported to be dependent on the composition of the must and on fermentation conditions (Schreirer, 1979). In other words, most of the fatty acids in wine are mainly produced by fermentation (Li et al., 2005). In general, the total content of acids was low in the four wines. The formation of volatile organic acids during yeast fermentation is quantitatively small, but it cannot be neglected from the viewpoint of flavor (Hernanza et al., 2009). Acetic acid is produced during alcoholic and malolactic fermentation. At low levels, this compound lifts the flavor of the wine, while at high levels, it is detrimental to the taste of wine because it causes the wine to taste sour and thin (Joyeux et al., 1984). In this study, it decreased gradually with time. Acetic acid, hexanoic acid, and octanoic acid were found in all four wines, 2-methyl-propanoic acid and dodecanoic acid were found only in the 2006 wine, while n-decanoic acid only in the 2009 wine, respectively. These C_6 to C_{10} fatty acids at concentrations of 4 to 10 mg/L impart a mild and pleasant aroma to wine; however, at levels beyond 20 mg/L, their impact becomes negative (Shinohara, 1985). The C_6 to C_{10} fatty acids did not have a significant impact on the aroma of the four wines examined in the current study because their levels were all far below 4 mg/L (Zhang et al., 2007).

Aldehydes and ketones

Carbonyl compounds primarily include aldehydes and ketones, most of which are produced by microbial activity. These compounds can impart a more rich, elegant, and unique aroma to wine (Li et al., 2005). Nonanal, furfural, and 5, 2-methyl-furan carboxaldehyde were unique aldehydes of the 2009 wine, acetoin was a unique ketone of the 2008 wine, while benzaldehyde was absent in the 2006 and 2008 wines. Other compounds isolated from the four wines included 3-furaldehyde and methoxy-phenyl-oxime.

Variation of aromatic compounds in the four wines

In the general analysis of the number and quantity variation of aromatic compounds in the four wines (Figure 1), the compounds that changed significantly were alcohols and esters, which led to the variation of the sum of the aromatic compounds. During storage, the sum of all the individual aroma compounds studied increased progressively despite a slight decrease during the initial stage that was attributable to the loss of alcohols. Acids, aldehydes, ketones, and other aroma compounds increased, though not significantly. Thus, the profiles of all the aroma compounds for cabernet sauvignon wine became increasingly diverse.

Variation of aroma descriptor groups

Aroma compounds play an important role in the quality of wine because they produce an effect on the senses (Vilanova et al., 2010). The aroma of wine is normally

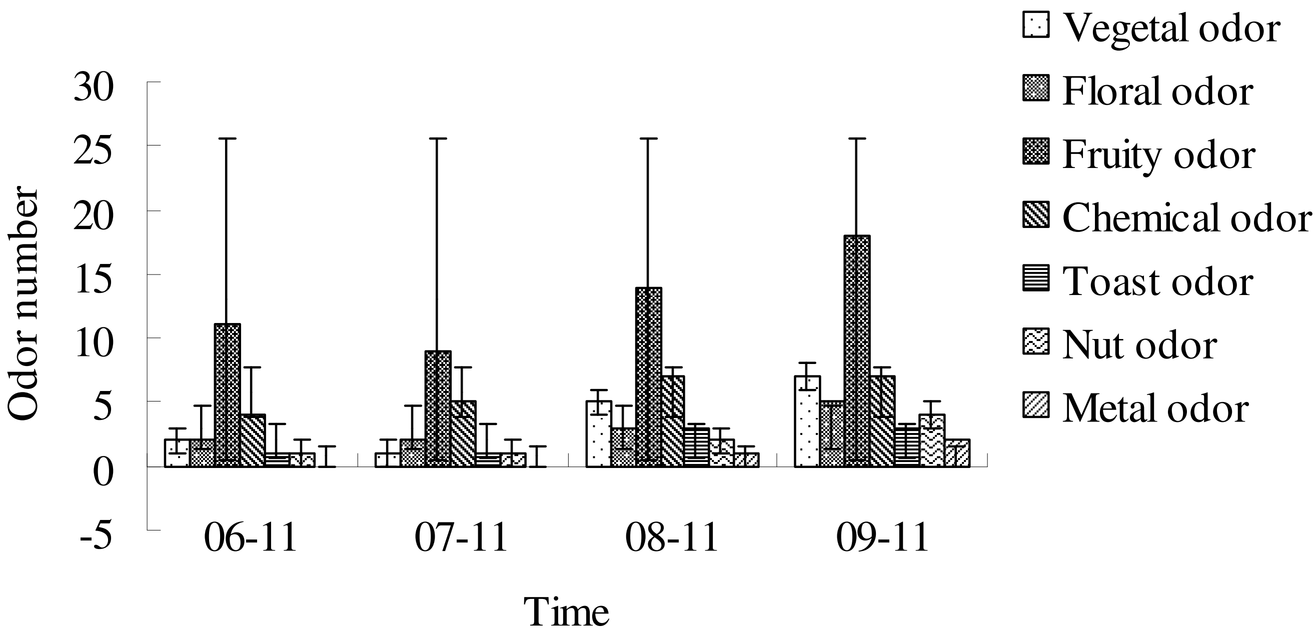

Figure 2. Variation of aroma descriptor groups.

produced by a specific ratio or combination of a multitude of volatile compounds (Juanola et al., 2004). The four wines were evaluated by sensory descriptive analysis to obtain the aromatic descriptors. Descriptive analysis revealed that the four wines were characterized by aroma descriptors belonging to six groups: vegetal, floral, fruity, chemical, toast, nut and metal odor (Figure 2).

The results (Figure 2) of the analyses indicated that the compounds that most contributed to the flavor of the four wines were fruity (1-propanol, 1-octanol, ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and octanoic acid) and chemical (ethyl alcohol, 1-butanol, 2, 3-butanediol, and hexanoic acid) aromas. On the other hand, Vilanova et al. (2010) reported that the compounds that most contributed to the flavor of Spanish Albariño wines were fruity and floral aromas. Figure 2 therefore indicate that fruity, floral, and chemical were the aroma descriptor groups that changed significantly as observed through analysis of the geometric mean and standard deviation.

Conclusion

This work aims to improve the understanding of the influence of storage in stainless steel tanks on the aroma compounds of cabernet sauvignon during the aging period. The study demonstrates the component and modification characteristics of the aroma compounds derived from four wines with different maturity. The ability to distinguish the aroma of the four wines was probably due to the dominance of alcohols, ethyl esters of fatty acids, and their contributions to the global aroma.

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