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Research Article

Influence of Low Temperature Enzyme Maceration Techniques on Volatile Compounds of Semi-dry Wine Made with cv. *Premier* of Rabbiteye Blueberries (*Vaccinium ashei)*

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Abstract: A low temperature enzyme maceration treatment was conducted during fermentation process of semi-dry cv. *Premier* blueberry wine. The aim of this study was to investigate the influence of maceration conditions on the wine aroma. As a pre-treatment, blueberry must was divided into 6 samples which were respectively treated by pectinase with 6 different maceration conditions at 6°C 1day, 6°C 2 days, 6°C 3 days, 16°C 1 day, 16°C 2 days and 16°C 3 days. After that wines were obtained by fermentation with *Saccharomyces cerevisiae*. Volatile compounds of wines were analyzed by GC-MS. Overall, the typical aroma compounds of semi-dry cv. *Premier* wines were constituted by three groups of organic compounds including esters, alcohols and fatty acids. Isoamyl acetate, ethyl caprylate, ethyl decanoate, 2-phenethanol and 3-methylbutanoic acid, which occupied 60% of the typical volatile aromatic compounds, all had higher Odor Activity Values (OAVs) in 6°C 3 days than other conditions. Maceration temperature and time had a significant effect on concentration and varieties of wines aroma substances. The results presented will help to better understand the aroma winemaking potential of this variety.

Keywords: Cv. *premier* blueberry, enzyme maceration, low temperature, OAVs, semi-dry wine, volatile compounds

INTRODUCTION

Blueberry belongs to the *Vaccinium* family. The fruit of the blueberry has a good taste and it has been widely used to make jam, juice and wine in the market. Rabbiteye blueberry (*Vaccinium ashei*) is a type of blueberry that famous for its high antioxidant activity and radical scavenging capacity and is valued for their thick skin and high phenolics (Wang *et al*., 2011; Su and Chien, 2007). Cv. *Premier* is one of the rabbiteye cultivars, which has been widely cultivated in China.

According to recent studies, Flavor of the wine has been found to be an important aspects when consumers consider purchasing wines (Butkhup *et al*., 2011). Wine aroma and flavour are composed of hundreds of volatile chemical compounds generating from the berry, wine-making and aging process (King *et al*., 2010). High-class wines have complicated mutual effect between the volatile aromatic compounds.

Harvesting fruit at specific stages of ripeness decides the style of wine. Once harvested, specific processing techniques and fermentation strategies will further determine the aroma and flavor development of the wine. Some studies have been carried out to determine the volatile compounds contributing to various character of wine (San-Juan *et al*., 2011; Escudero *et al*., 2007). Over 800 aroma compounds have been identified in wine. The combination of them forms the character of wine and differentiates one wine from another (Komes *et al*., 2006).

Wine aroma was affected by various factors. Some influencing factors, such as pre-fermentative maceration, wine yeast strains and application of malolactic fermentation, would make a big difference during vinification (Styger *et al*., 2011). Enzyme-treated wines show significant improvement which terpenes and 2-phenylethanol concentration increased in four white varieties (Valcárcel and Palacios, 2012). Strain with higher nitrogen demand produces higher content of esters during wine fermentation while the concentration of alcohols decreases (Torrea *et al*., 2002).

The reasonable choices of temperature and skin contact time are important factors to be considered in the low temperature enzyme maceration, for example, color quality is improved with lower temperature (Gómez-Plaza *et al*., 2000), polysaccharide and proanthocyanidin concentrations increase during longer maceration when color and anthocyanin concentration do the opposite (Gil *et al*., 2012). Enzyme maceration treated wines can give a promotion of polymeric pigment, but less monomeric anthocyanins than non-enzyme-treated wines (Parley *et al*., 2001). The concentration of esters and terpenols would increase in

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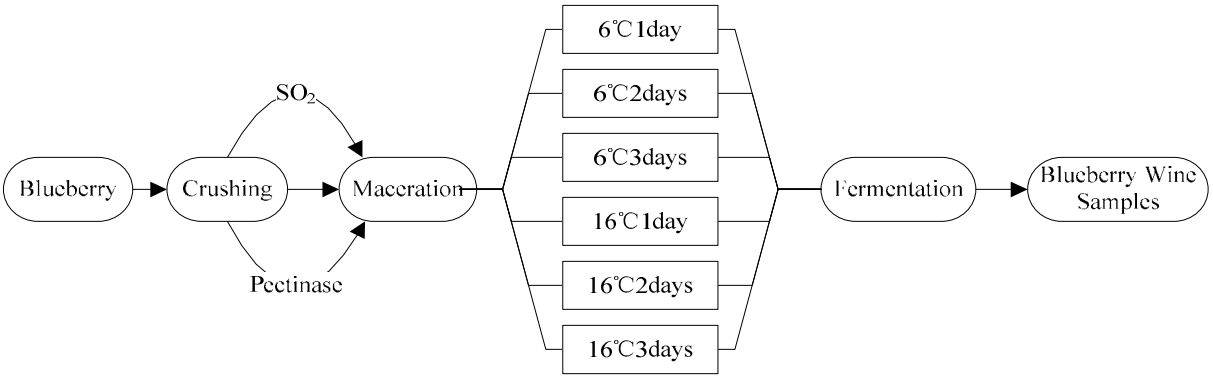


Fig. 1: Winemaking process flow path chart

wine with low-temperature prefermentative maceration process (Salinas *et al*., 2003). So the maceration condition is considered to be one of the major factors of the wine quality.

No previous report is seen regarding the aroma components analysis on blueberry wine and the influence on blueberry wine aroma quality caused by different fermentation strategies. The present work is to monitor the volatile aromatic components in semi-dry cv. *Premier* blueberry wine made in different low temperature enzyme maceration techniques and to choose a better process and optimize the brewing technology of blueberry wine.

MATERIALS AND METHODS

Blueberry samples: All experiments were performed in triplicate. Fresh cv. *Premier* of rabbiteye blueberries were obtained during a harvest in 2011 from blueberry plantation located in the central-eastern of China. A portion of the sample was analyzed immediately for oenological parameters, while the remainder was frozen and stored at -20°C for subsequent making wine. Reducing sugar concentration of the berry was 99.8 g/L, The pH was 3.6 and the concentration of titratable acid was 7.2 g/L in the berry.

Wine samples: The berries were crushed into mash and then treated with 0.2 g/kg pectinase (Lafase He Grand Cru from Laffort) and 60 mg/L SO2. The prepared mash was divided into 6 flasks (500 mL) equally. Six samples were separately treated with low temperature maceration condition of 6°C 1 day, 6°C 2days, 6°C 3 days, 16°C 1 day, 16°C 2 days and 16°C 3 days. Once maceration step was completed, the crushed blueberry masses underwent the following process. After maceration, the wine samples were adjusted to 180.0 g/L of fermentable sugar with sugar and added 0.2 g/L yeast, which was activated by blueberry juice for about 15 min at 35°C. Then the main fermentation was carried out at 20°C and ended when the sugar content was below about 10.0 g/L. After centrifugation (4000 rpm) and press, the wine samples were carried out fining separation and sediment removal. Then the wines were sealed and stored at 16°C for one week before being proceeded with preliminary treatment for GC-MS. Winemaking process flow path chart was presented in Fig. 1.

Instruments and reagents: GZX constant temperature incubator was purchased from Jintan Experiment Instrument Factory, China. DL-5M low-speed freezing centrifuge was purchased from Changsha Xiangyi Centrifuge Instrument Co., LTD, China. GCMS-QP 2010 gas chromatography-mass spectrometry was purchased from Shimadzu Corporation, Japan. Analytical grade of isoamyl acetate (98.0%), ethyl caprylate (99%), ethyl hexanoate (≧98%), ethyl lactate (98%), decanoic acid (99%) and 3-methylbutanoic acid (99.5%) were purchased from Shanghai Sinopharm Co., LTD, China. 2-octanol (≧99%) as an internal standard, ethyl decanoate (99%) and 2-phenylethanol (98%) were purchased from Aladdin chemistry Co., LTD, China. Solvent of dichloromethane (≧99.5%) and anhydrous sodium sulfate were purchased from Shanghai Sinopharm Co., LTD, China. A mixture of a series of C_8-C_{20} was purchased from Sigma-Aldrich Trading Co., LTD, Shanghai.

Liquid-liquid extraction of wine volatile compounds: Wine samples (100 mL) were extracted three times by shaking with 1 mL of internal standard (a solution of 41.75 ug/mL 2-octanol in dichloromethane) and 50, 30, 30 portions of dichloromethane. The extracts were mixed and dehydrated with anhydrous sodium sulfate. Then the dichloromethane extracts were stored at -5°C for 24 h in order to separate the frozen water from the organic phase. After 24 h, the extracts were slowly concentrated to 2 mL and then to 1 mL with a rotary evaporator at 0~5°C. The concentrate (1 mL) was stored in a glass screw-top vial at 5°C.

GC-MS analysis condition: The extracts were analysed by GC-MS on SHIMADZU GC-MS-QP 2010 gas chromatography-mass spectrometry, equipped with

a 30 m×0.25 mm (i.d.), 0.25 μm coating thickness DB-5 fused silica capillary column. The carrier gas was helium at 1 mL/min and split ratio was 30:1. The temperature program was kept at 40°C for 2 min and raised to 180°C at 4°C/min and then raised to 250°C at 10°C/min for 6 min. The transfer line temperature was 230°C and the injection port temperature was 250°C.

Compound identification: The mass spectrometric data of GC-MS analysis was got from the standard database of NIST147, NIST27 and WILEY7. The identification of volatile compounds was confirmed by injection of pure chemicals as reference for retention times and comparison of their Retention Indices (RI), determined by using an alkane standard mixture (C_8-C_{20}). The pure chemicals and n-alkanes were injected at the same condition as the samples.

Classic enological parameters: Reducing sugars, pH and total acidity were measured according to International Organization of Vine and Wine (O.I.V.) methods.

RESULTS AND DISCUSSION

The GC-MS analysis results of six wine samples are shown in Table 1, average concentration of the extracted aroma substances and thresholds of certain

Table 1: Average concentration (mean±standard deviation) and Odor Perception Thresholds (OPT) of volatile compounds determined in semi-dry rabbiteye blueberry wines

		Concentration (ug/L)						
Compound	RI	6°C 1day	6° C2days	6°C3days	16°C1day	16°C2days	16°C3days	OPT (ug/L)*
Esters								
Ethyl lactate[ab]	805	490±51	673±52	504±67	462±49	492±32	358±53	250, 000
Isoamyl acetate[abc]	873	450±39	498±53	738±29	528±16	642±45	501±30	160
γ-Butyrolactone[bc]	907	152±22	180±18	229±27	148±31	193±37	147±16	1, 000
3-Hydroxy-Butanoic acid. ethyl ester[b]	932	33±12	66±3	49±5	-	53±6	35±2	20, 000
2-Hydroxy-3-methyl-Butanoic acid[b]	963	-	9±2	-	-	-	-	-
Ethyl hexanoate[abc]	999	368±57	330±16	495±36	337±26	508±99	439±44	14
Pantolactone[b]	1039	53±8	-	-	58±9	71±12	65±11	-
Oxalic acid, isobutyl nonyl ester[b]	1048	-	-	-	-	21±4	-	-
4-Hydroxybutanoate ethyl[b]	1055	840±86	1093±25	2072±123	1114±168	1245±167	942±88	-
Ethyl caprylate[abc]	1198	400±28	399±25	610±72	456±72	516±37	372±22	240
2-phenylethyl acetic acid ester [b]	1256	72±10	69±4	117±13	80±5	80±9	151±21	5,000
Ethyl 9-decenoate[b]	1389	92±15	132±17	101±10	-	-	35±7	-
Ethyl decanoate[abc]	1397	129±11	86±7	215±44	187±14	186±41	148±28	200
Ethyl laurate[bc]	1596	-	-	-	80±6	78±4	63±8	40
Hexadecanoic acid, ethyl ester[bc]	1998	56±4	330±13	22±6	136±12	23±5	17±4	-
Alcohols								
2,3-Butanediol [bc]	nm	7892±107	16006±84	13913±122	8343±86	12064±124	11060±84	150, 000
1-Hexanol[bc]	865	-	74±7	-	51±8		74±16	8, 000
3,3-Dimethyl-2-butanol[b]	940	-	-	-	-	34±6	-	50, 000
3-Methyl-1-hexanol[b]	969	-	-	-	36±7	-	-	200, 000
1-Heptanol[bc]	970	-	75±5	-	-	-	-	-
3-(methylthio)-1-Propanol[b]	977	645±35	870±19	1185±39	468±51	766±103	334±68	1,000
2,7-Dimethyl-4,5-Octandiol[b]	1025	-	50±2	34±5	-	32±9	21±5	-
Benzyl alcohol[bc]	1034	-	-	-	16±4	-	22±7	100, 000
2-Phenethanol[abc]	1102	18739±292	21967±179	28575±264	17645±187	17689±166	11449±73	10, 000
2-6-Dimethyl- 7-Octene-2-6-diol[b]	1231	12±3	22±5	26±6	-	-	-	-
4-Hydroxy- benzeneethanol[b]	1427	830±14	1093±38	1790±48	904±71	1602±25	1669±38	-
Acids								
3-Methylbutanoic acid[ab]	835	140±8	172±10	188±19	52±3	102±10	127±12	33
2-Methylbutanoic acid[bc]	845	111±10	152±21	169±37	88±10	107±9	109±14	50
Hexanoic acid[bc]	984	904±13	830±17	925±33	1010±25	838±53	666±37	3, 000
Octanoic Acid[bc]	1179	2507±145	1195±21	3725±59	2962±84	3810±107	2537±66	10, 000
9-Decenoic acid[bc]	1362	404±41	859±64	491±28	190±32	460±55	258±21	40
Decanoic acid[abc]	1369	687±29	709±23	1313±56	1074±73	139±26	1064±65	1, 400
Quinic acid[b]	1671	-	72±18	186±24	-	-	-	-
Others								
2H-Pyran-2,6(3H)-dione[b]	991	161±11	156±33	74±8	77±15	628±122	87±7	-
4, 4-Dimethyl-3-hydroxy-3[2H]-furanone[b]	1040	-	56±4	77±9	-	-	-	-
Dihydro-5-(1-hydroxyethyl)-2(3H)-furanone[b]	1217	-	-	-	38±6	-	-	-
4-ethenyl-2-methoxy phenol[b]	1311	63±2	105±11	113±12	106±21	-	-	380

*: Odor perception thresholds (OPT) reported in the literature (Salinas *et al*., 2003; Aznzr *et al*., 2003; Angioni *et al*., 2012; Chaves-Lopez *et al*., 2009; Mingorance-Cazorla *et al*., 2003; Viana *et al*., 2009; Pla *et al*., 2003; Rocha *et al*., 2003; Lerma *et al*., 2012; Krist *et al*., 2004; Santos *et al*., 2004); [a]: Identification by injecting reference substances; [b]: Identification by GC-MS by comparing with the standard database; [c]: Retention index reported in the literature (Jorge *et al*., 2005; Xu *et al*., 2007; Willner *et al*., 2013; Poisson and Schieberle, 2008; Barros *et al*., 2012); nm, not measured, beyond C_8-C_{20}

Table 2: Mean Odor Activity Values (OAVs) and odor descriptor of typical aroma compounds

	Odor activity values (OAVs)						
Compound	6°C 1day	6°C 2days	6°C 3days	16°C 1dayC	16°C 2days	16°C 3days	Odor perceived*
Isoamyl acetate	2.83	3.11	4.61	3.30	4.02	3.13	Banana, fruity, sweet
Ethyl caprylate	1.66	1.65	2.54	1.90	2.15	1.55	Ripe fruits, pear, sweety
Ethyl hexanoate	26.27	23.55	35.37	24.07	36.28	31.37	Fruity, strawberry, apple
γ-Butyrolactone	0.15	0.18	0.23	0.15	0.19	0.15	Toasted, rubber
Ethyl decanoate	0.64	0.43	1.07	0.93	0.93	0.74	Fruity, soapy
Phenethyl acetate	0.01	0.01	0.02	0.02	0.02	0.03	Rose, honey
Ethyl laurate	-	-	-	2.00	1.95	1.58	Oily, fatty, floral
2-Phenethanol	1.87	2.20	2.86	1.76	1.77	1.14	Rose, honey
2,3-Butanediol	0.05	0.11	0.09	0.06	0.08	0.07	Floral, fruity, herbal
Octanoic acid	0.25	0.12	0.37	0.30	0.38	0.25	Fatty acid, rancid, dry, dairy
Hexanoic acid	0.30	0.28	0.31	0.34	0.28	0.22	Cheese, fatty, grass, fruity
Decanoic acid	0.49	0.51	0.94	0.77	1.00	0.76	Fatty acid, rancid, dry, woody
9-Decenoic acid	10.09	21.48	12.28	4.75	11.48	6.45	Waxy, fatty, soapy
3-Methylbutanoic acid	4.19	5.15	5.62	1.56	3.06	3.79	fatty, rancid and cheesy
4-ethenyl-2-methoxy phenol	0.17	0.28	0.30	0.28	-	-	Black pepper, species, clove-like

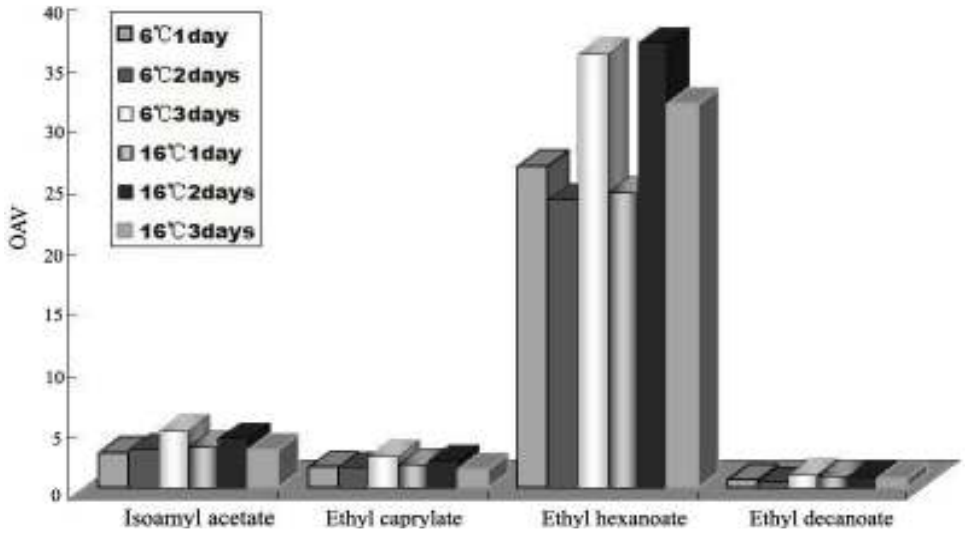
*: Odor descriptor reported in the literature (Salinas *et al*., 2003; Aznzr *et al*., 2003; Angioni *et al*., 2012; Chaves-Lopez *et al*., 2009; Mingorance-Cazorla *et al*., 2003; Viana *et al*., 2009; Pla *et al*., 2003; Rocha *et al*., 2003; Lerma *et al*., 2012; Krist *et al*., 2004; Santos *et al*., 2004)

compounds are listed. It can be seen from the Table 1 that the aroma substances content and varieties of wine samples varied greatly in different cold immersing temperatures and time. The aroma extracts of blueberry wine mainly consisted of Ester, Alcohol and Fatty Acid. The typical aroma components identified included isoamyl acetate, ethyl caprylate, ethyl hexanoate, butyrolactone, ethyl decanoate, phenethyl acetate, ethyl lactate, ethyl laurate, phenylethyl alcohol, 2, 3-butanediol, octanoic acid, hexanoic acid, decanoic acid and 9-decenoic acid (Table 2), which were identified with a probability higher than 85% in all replicates.

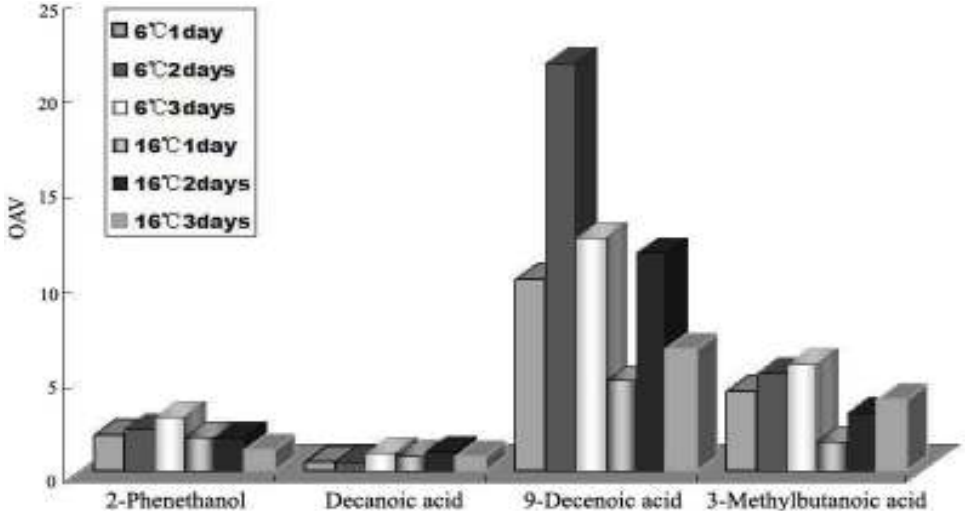
Most of the compounds identified in this study were also found in relevant studied wines (Aznzr *et al*., 2003; Angioni *et al*., 2012). Compared with the wine samples after the low-temperature treatment at 6°C 1 day, 6°C 2 days and 6°C 3 days, the blueberry wine samples after the treatment at 16°C 1 day, 16°C 2 days and 6°C 3 days can be detected with volatile aroma components including pantolactone, ethyl laurate, 4-hydroxybutanoate methyl, 2-phenylethyl acetic acid ester and benzyl alcohol, which have not been identified in the low-temperature treatment process at 6°C. Five compounds were only identified in the samples at 6°C, which included 2-hydroxy-propanoic acid ethyl ester, 2-6-dimethyl-7-octene-2-6-diol and quinic acid. As a result, pre-treatments at 6°C and 16°C both had their own characters and advantages. In addition to esters, alcohols and fatty acids, the two other chemical substances including 2 h-pyran-2, 6 (3 h)-dione and 4-ethenyl-2-methoxy phenol have been detected in all wine samples. But the difference of these two low-content chemical compounds was not obvious among 6 wine samples. In order to choose a better maceration condition, the analysis was based on the concentration of the aroma substances and relevant OAVs.

OAVs are calculated to evaluate the influence of low temperature maceration on wine aroma. The OAV is obtained as the ratio of compound concentration to its Odor Perception Threshold (OPT) value, which is to assess the influence of the single aromatic compounds produced during maceration (Chaves-Lopez *et al*., 2009). The OAVs of aroma components and sensory characteristics of six samples played an important role in reflecting the aroma typicity of cv. *Premier* blueberry wine and gave the wine distinctive style and its feature (Table 2). On the basis of odor description and threshold, the most powerful odorants were established and only those displaying OAVs greater than 1 were deemed to contribute to wine aroma (Mingorance-Cazorla *et al*., 2003). In this study only the main aroma compounds at OAVs values ≥1 were considered. OAVs (OAVs≥1) of main esters, alcohols and fatty acids in 6 samples are shown in Fig. 2.

Esters: Ester compounds were the most abundant chemical substances in all samples, including isoamyl acetate, ethyl caprylate, ethyl hexanoate, butyrolactone, ethyl decanoate and ethyl laurate (Table 2). Isoamyl acetate, which contributes with fruity notes to the wine, has a kind of banana flavor. The content of isoamyl acetate was up to 0.7 mg/L (Table 1). The similar experimental figure of such high content has been found in several literatures about wine aroma (Viana *et al*., 2009; Pla *et al*., 2003). Among the six wine samples, the OAV of Isoamyl acetate showed the highest activity in the 6°C 3 days, reached to 4.6 (Fig. 2). Ethyl caprylate with the aroma characteristics of ripe fruits, pear and sweety, also gives blueberry wine fruity flavor (Rocha *et al*., 2003), its OAVs were between 1.55 and 2.54 and also was the most outstanding in 6°C 3 days. Ethyl hexanoate (strawberry and apple aroma characteristics, the OAVs of which were between 26 and 36 in the six samples, showed the highest odor activity among all the volatile compounds. Especially in the sample after 16°C 2 days treatment, the OAV (36.3) was the highest of all the compounds (Fig. 2) and it reached to 35.4 in the 6°C 3 days sample.



(a): Main esters



(b): Main alcohols and fatty acids

Fig. 2: Odor activity values (OAVs≥1) comparison of main esters, alcohols and fatty acids in 6 samples

The aroma of γ-Butyrolactone is of burnt smell type and it is associated with a toasted and rubber descriptor (Lerma *et al*., 2012; Krist *et al*., 2004).

Ethyl decanoate is the fruital ester series (Santos *et al*., 2004). The OAVs was at a low level. Ethyl laurate has only been detected in the wine sample macerated at 16°C 1 day, 16°C 2 days and 16°C 3 days. The OAV of Ethyl laurate was almost at the same level among three 16°C wine samples while this compound can not be detected in the 6°C wine samples. Thus pre-treatment temperature may be one of the main influencing factors on ethyl laurate in the blueberry wine. Ethyl laurate pertains to oily, fatty, floral aroma series (Butkhup *et al*., 2011), which bring delightful flowery and fatty odor to blueberry wine.

In conclusion, the fruity aroma of cv. *Premier* semi-dry blueberry wine showed the most intense and outstanding after 6°C 3 days maceration treatment by analyzing the content, aroma type and OAV of esters. Due to the effect of ethyl laurate, blueberry wine would have advantages of the flowery and fatty odor when 16°C maceration treatment was taken.

Alcohols: The main alcohol aroma compounds included 2-phenylethanol, 2, 3-butanediol, 4-hydroxy-benzen-eethanol and 3-(methylthio)-1-propanol. The chemical substances had low OAVs which were all under 1 except 2-phenylethanol (Fig. 2). 2-phenylethanol played a role in the typical aroma of cv. *Premier* blueberry wine. 2-phenylethanol belongs to aromatic alcohol. It has a rose-like odor above its perception threshold value (Selli *et al*., 2003). According to determination by GC-MS, the concentrations of 2-phenylethanol found in extracts

were on the same order of magnitude as the values found for other wines (Fretz *et al*., 2005; Jurado *et al*., 2008; Pinillos *et al*., 2004; Gamero *et al*., 2011), had a concentration range from 11~28 mg/L and OAV from 1.1~2.8 (Table 1 and 2). The OAVs of 2-phenylethanol from 6°C wine samples all showed higher OAVs than 16°C wine samples. Because of its ability to produce strong flavor, 2-phenylethyl alcohol may be related to a higher capacity to utilise the assimilable nitrogen in must (Torrea *et al*., 2002). Maceration temperature has a great effect on the leaching of 2-phenylethanol precussor substances. Lower temperature pre-treatments bring about higher OAVs of 2-phenylethanol.

Fatty acids: Most of the organic acids in blueberry wine are produced by fermentation. The main volatile fatty acids (OVAs≥1) in blueberry wine were decanoic acid, 9-decenoic acid and 3-methylbutanoic acid. decanoic acid plays a role in herbaceous and fatty odor series. It showed higher OVAs (0.94 and 1.0, respectively) in 6°C 3 days and 16°C 2 days sample than the others. The concentration of 9-decenoic acid was detected to be the highest of all fatty acids aroma compounds. The presence of 9-decenoic acid in rabbiteye blueberry wine has been described with waxy, fatty and soapy, which belong to fatty odor (Butkhup *et al*., 2011). Figure 2 indicated that 9-decenoic acid OAVs in 6°C 1 day, 6°C 2 days and 6°C 3 days (10.09, 21.48 and 12.28, respectively) all had higher activity than 16°C 1 day, 16°C 2 days and 16°C 3 days sample (4.75, 11.48 and 6.45, respectively). 9-decenoic acid aroma value reached its highest in 6°C 2 days of all samples. The same situation happened to 3-methylbutanoic acid (fatty) OVAs had a range from 4~6 in 6°C samples while from 1~4 in 16°C samples. 6°C 3 days had the highest aroma value of 3-methylbutanoic acid in all the samples. 3-methylbutanoic acid was found to be very intense at the sniffing port and its odor descriptors were fatty, rancid and cheesy (Gil *et al*., 2012). From what have been discussed above, fatty acids samples had higher OAVs when macerated in a temperature of 6°C than 16°C.

CONCLUSION

It can be concluded that, isoamyl acetate, ethyl caprylate, ethyl hexanoate, ethyl decanoate, ethyl lactate, phenylethyl alcohol, decanoic acid, 9-decenoic acid and 3-methylbutanoic acid played an important role to bring the blueberry wine with strong typicity. The results suggested that maceration temperature and time had a significant effect on concentration and variety of aroma substance. Only wine under 16°C did have ethyl laurate which was described by floral and fatty odor, while maceration condition of 6°C was adverse to the production of it. However, 60% of the typical volatile aroma compounds, including isoamyl acetate, ethyl caprylate, ethyl decanoate, 2-phenethanol and 3-methylbutanoic acid, had the highest OAVs when maceration condition at 6°C 3 days. The semi-dry cv. *Premier* blueberry wine macerated at 6°C 3 days was identified to be more abundant in volatile aroma compounds.

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