Effects of non-Saccharomyces Cerevisiae Hanseniaspora Uvarum on Wine Fermentation

Xiaojian Tang [1], Xi He [1], Yuxia Sun [2]

[1] School of Bioengineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, Shandong, China

[2] Grape Research Institute, Shandong Academy of Agricultural Sciences, Jinan 250100, Shandong, China

* Corresponding Author Email: hx@qlu.edu.cn

Abstract. Single strain fermentation with simple active dry yeast is easy to cause wine homogenization. Saccharomyces cerevisiue and non-saccharomyces cerevisiue mixed fermentation have different effects on wine flavor. In this study, Hanseniaspora uvarum Hu2010 isolated from wild grape juice was taken as the object, and Saccharomyces cerevisiue D254 was used for single fermentation and mixed fermentation respectively. The influence of different yeast fermentation processes on wine aroma was studied by analyzing the physical and chemical indicators such as sugar and organic acid content in the fermentation process, volatile aroma components and sensory evaluation. The results showed that the alcohol content of the three groups was similar, but the total sugar, revertose and total acid content of the wine samples inoculated with Hanseniaspora uvarum Hu2010 were significantly higher than those of the other two groups. The results of the gas chromatography-mass spectrometry analysis showed that Hanseniaspora uvarum Hu2010 could increase the content of multiple esters in the wine sample compared with D254 fermentation alone. In addition, the total acceptability of Hu2010 was comparable to that of S. cerevisiae D254 regardless of fermentation alone or mixed fermentation, but the scores of floral and fruity flavors were higher. It indicates that Hu2010 plays a positive role in wine aroma.

Keywords: Mixed fermentation, Wine, Saccharomyces cerevisiue, Hanseniaspora uvarum, wine Aromas.

1. Introduction

The quality of wine is mainly determined by the composition and concentration of aroma [1], There are three main sources of aroma: the variety aroma of grape fruit, fermentation aroma produced by microbial metabolism and aging aroma, among which fermentation aroma is the most important [2]. The production of aroma is the result of the joint action of different types and quantities of yeast, which directly determines the aroma and taste of wine [1]. At present, commercial Saccharomyces cerevisiae is mostly used in wine brewing, and there is a lack of yeast with regional characteristics. Therefore, many studies have screened excellent yeast strains from the natural fermentation process of grapes in the producing area to brew wine with local characteristics [3].The natural fermentation of wine is mostly caused by Non-Saccharomyces wine yeast [4], Non-Saccharomyces cerevisiae can increase the content of grade alcohols and esters, and increase the pleasant aroma of flowers and fruits in wine [5], Mixed fermentation with Saccharomyces cerevisiae can improve the organoleptic quality of wine [6]. Among them, Hanseniaspora uvarum has a good ability to produce acetate and is the main aroma component of white wine [7]. Mixed fermentation of Kluyveromyces thermotolerant and Saccharomyces cerevisiae to reduce acid content in wine [8]; Using Maggi Mage and Saccharomyces cerevisiae to reduce the alcohol content of ordinary wine by 1.6% [9].

Petit Manseng, whose main origin is located in the Pyrenees-Atlantic department in southwestern France, although the wine in this region has smaller fruit grains, relatively thick skin and relatively low yield, its brewing The resulting wines are aromatic, late-ripening, high in sugar, acidity, and quality; the most pronounced aromas in wines made from Petit Munson are peach, honey, and cinnamon, and sometimes tropical fruit. Flavor, such as passion fruit, pineapple, etc [10]. In this study, the grape variety Petit Munsen was used as raw material, and a non-Saccharomyces cerevisiae strain, Hanseniaspora uvarum Hu2010, which was screened in the previous study, was used for pure and mixed fermentation with commercial Saccharomyces cerevisiae D254, respectively, to explore the effect of different yeast strains on the composition and aroma of wine.

2. Materials and Methods

2.1. Raw materials, strains and culture medium

Petit Manseng grapes, the grape juice has been pressed, stored in the refrigerator, the refractive sugar is about 20.86°brix, pH=3.2; commercial active dry yeast D254, purchased from Shanghai Jietu Industry and Trade Co., Ltd.; non-Saccharomyces cerevisiae: (The isolated and purified grape juice Hanseniaspora uvarum Hu2010) was preserved by the laboratory of Shandong Academy of Agricultural Sciences; YPD medium: yeast extract 10g/L, protein 20g/L, glucose 20g/L, agar 20g/L (solid medium supplement), Sigma Company, USA; 4-methyl-2-pentanol chromatographically pure (98%), Aldrich Company, USA.

2.2. Reagents and Ritual Equipment

Vitamin C; sodium hydroxide standard titration solution (0.05mol/L), phenolphthalein reagent (10g/L), hydrochloric acid solution, sodium hydroxide solution (200g/L), glucose standard solution (2.5g/L), methine Blue indicator solution (10g/L), Fehling solution, Sinopharm Chemical Reagent Co., Ltd.; GCMS-QP2020 Gas Chromatography Mass Spectrometer Automatic Solid Phase Microextraction (SPME) Sampler (Shimadzu); 50/30μm DVB/CAR/PDMS extraction head, DB-WAX capillary column (30m*0.25mm*0.25gym): American J&W company

2.3. Experiment method

1.3.1 inoculation and fermentation

Three different yeasts were separately inoculated into the prepared fresh YPD liquid medium, and activated to obtain seed liquid. The absorbance value (OD600) at 600 nm was determined by UV spectrophotometry. When OD600=1.0, the yeast concentration is about 10^6CFU, and the inoculum is calculated based on this. Prepare 9 1000ml glass bottles, sterilize them with alcohol, number from 1 to 9, transfer the grape juice into the glass bottles by siphon (avoid sucking the bottom sediment), each bottle is about 500ml. Yeast inoculation was started on a sterile clean bench at a concentration of 10^5CFU.Bottles 1-3 were inoculated with Saccharomyces cerevisiae D254; bottles 4-6 were inoculated with non-Saccharomyces cerevisiae (Hanseniaspora uvarum) Hu2010; bottles 7-9 were inoculated with mixed yeast (1:1 mixture of D254 and Hu2010). Fermentation was carried out in an environment of about 20°C, during which the absorbance (OD value) was measured every day to observe the growth activity of yeast.

1.3.2 Determination of physical and chemical indicators

The content of total sugar, total acid, alcohol content, etc. is determined with reference to the national standard GB/T15038-2006 "General Analysis Method for Wine and Fruit Wine".

1.3.3 GC-MS Analysis of Volatile Compounds in Wine

GC-MS is a combination of gas chromatography separation and high sensitivity of mass spectrometry. GC has a strong separation ability, while MS contains element information, molecular weight, and molecular structure data, which can obtain both the retention time and molecular structure of the component to be tested. The instrument analysis system is widely used in safety fields such as environment and food and drug testing [11].

The experimental conditions of gas chromatography were as follows: the gas chromatography-mass spectrometer was a Shimadzu GCMS-QP2020 gas chromatography-mass spectrometer equipped with a PAL-SPME autosampler. The capillary column used was HP-INNOWAX

60m×0.25mm×0.25μm (J & WScientific, Folsom, CA, USA). The carrier gas is high-purity helium (purity 99.999%), flow rate: 1mL/min. Automatic injection. The heating program of the column oven: hold at 50°C for 1min, then heat up to 220°C at a rate of 3°C/min, hold for 5min, and the total running time is 62.67min. The mass spectrometer interface temperature was 280°C, the ion source temperature was 230°C, the quadrupole temperature was 150°C, the ionization method was electron bombardment ionization (EI), the ion source energy was 70eV, and the mass scanning range was 20-350m/z. Three independent replicates were performed for each sample.

The internal standard method was used for quantitative analysis, and the internal standard was 4-methyl-2-pentanol. The peak area integration adopts the ion mode (SIM), selects the characteristic ion of the quantitative compound, and integrates the peak area of the characteristic ion peak of the compound. The calculation formula is as follows: target compound concentration = target compound peak area/inner surface peak area × inner surface concentration (mg/L).

2.4. Sensory evaluation of wine

Sensory evaluation of wine samples by 10 tasters, no communication with each other during the evaluation. The wines are scored mainly from three aspects. First, the olfactory complexity and intensity are scored; and then four aromas are specified as floral, fruity, buttery, vanilla and ester. Score separately; then taste the taste, score the wine body, complexity, balance, and aftertaste (the full score of each sub-item is 10 points), and finally find the average value of each wine sample and make a radar chart.

3. Conclusion and Analysis

3.1. Effects of Different Bacteria Fermentation on Main Physicochemical Indexes of Wine

Table 1. Physical and chemical data of different yeast fermentation

strain	Total acid (g/L)	Total sugar (g/L)	Reducing sugar (g/L)	Alcohol content (%)
D254	8.62±0.35	3.88±0.39	3.65±0.35	11.51±0.29
D254+Hu2010	8.58±0.88	4.18±0.88	3.35±0.57	11.55±0.14
Hu2010	10.18±0.28	6.33±2.23	4.25±0.49	11.93±0.25

The three treatments were repeated three times each, and a total of nine groups of physicochemical data were compared.Comparing the total acidity of the three groups, it can be clearly seen from Table 1 that the total acid content of the three wine samples inoculated with Hanseniaspora uvarum 2010 is significantly higher than that of the other two groups, which shows that the use of non-Saccharomyces cerevisiae for fermentation is not effective for wine. The content of acid has a certain influence; and the total acidity of the first group of wine samples fermented only with Saccharomyces cerevisiae D254 is similar to that of the two kinds of bacteria mixed inoculated and fermented.

From the analysis of the total sugar and reducing sugar residue data, the same inoculation of HU2010 is higher than the other two groups in both total sugar and reducing sugar content. The sugar content is the lowest, and the reducing sugar content of the mixed inoculated wine samples is the lowest, but the numerical difference between the two groups of experimental data is not large. Therefore, it can be seen that the use and conversion of sugar substances in fermentation with non-Saccharomyces cerevisiae is still lower. in Saccharomyces cerevisiae. In addition, the alcohol content of the three groups of wine samples is very similar, almost all in the range of 11-12%.

3.2. Analysis of Aroma Substances

2.2.1 Esters

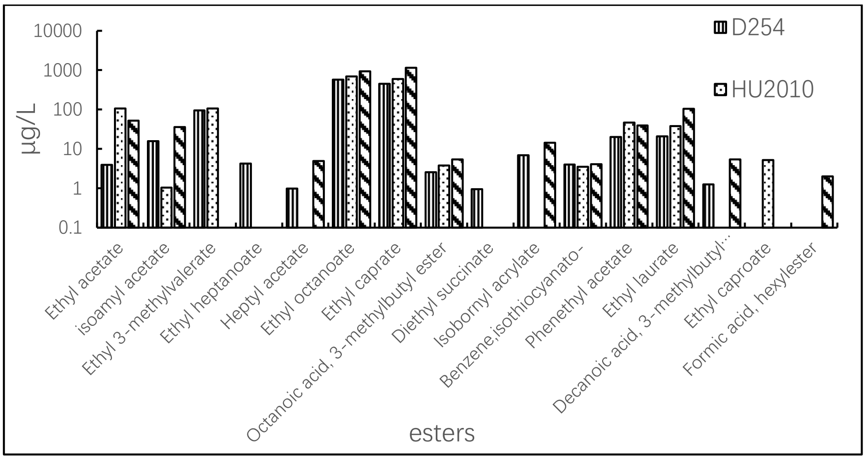

Figure 1. Ester content in different wine samples (μg/L)

Esters refer to substances that can directly produce important aromas such as floral and fruity aromas in wine [12]. Fermented esters play an important role in the fruit aromas in wine, especially in very young red and white wines [13]. Esters in wine are by-products of yeast fermentation, and the unique taste of esters similar to fruit makes wine produce some special aromas, such as banana, apple, pineapple, lemon, coconut and other smells.

Wine contains more ethyl acetate esters, which have a certain apple flavor; then there are ethyl caproate and ethyl caprylate. In addition to bringing more unique aromas to wine, esters are also considered to be a measure of wine quality and as an indicator of fermentation process control. There are two main ways to form wine esters, one is the synthesis of microorganisms (the main way) and the other is the esterification reaction during the aging process [14-18]. Different strains used in fermentation will also lead to certain differences in the content and composition of esters produced [19, 20].

In this test, 16 kinds of esters were detected in the three groups of wine samples, of which the content of ethyl caprylate and ethyl capric acid was relatively high. Ethyl esters have fruity, fatty and pleasant vinegar flavors; and the content of these two substances is the highest in the mixed fermentation of D254+Hu2010, but the total esters in this wine sample are the least. In addition, compared with the wine samples inoculated with Saccharomyces cerevisiae D254 alone, the wine samples inoculated with non-Saccharomyces cerevisiae, whether inoculated alone or in mixed fermentation, found unique esters in the two wine samples; for example, the wine samples inoculated with Hu2010 alone It contains ethyl n-hexanoate, which has a fruity aroma; while the wine sample mixed with D254+Hu2010 contains hexyl formate, which has a sweet aroma like apples and unripe plums, and has a corresponding sweetness. When tasting, this group of wines tasted a little sweeter than the other bottles.

2.2.2 Alcohols

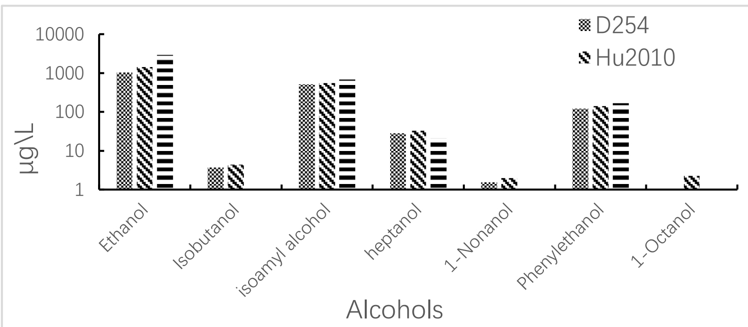

Figure 2. Alcohol content in different wine samples (μg/L)

Alcohols in wine mainly come from yeast alcoholic fermentation, amino acid conversion and oxidation of linolenic acid degradation products [21]. Alcohol compounds are the main products of wine yeast fermentation, and the produced alcohols are mainly ethanol and a small amount of other alcohols. Alcohol compounds have an important influence on the aroma of wine, and an appropriate amount of higher alcohol will bring good aroma to wine [22].

A total of 7 kinds of alcohols were detected in this experiment, and the content of each group was similar. Among them, the content of ethanol, isoamyl alcohol and phenethyl alcohol was higher, and isoamyl alcohol had bitter almond taste, which is not a pleasant wine for wine. On the palate, phenylethyl alcohol has a rose aroma and thus can impart a certain floral aroma to the wine. Compared with single fermentation, isobutanol, nonanol and octanol were not detected in the mixed fermentation group.

2.2.3 Aldehydes and ketones

Aldehydes often have a floral odor [23]. Although their content is very small, they may contribute a lot to the aroma of wine, so it cannot be analyzed purely from the content, but also the aroma threshold of different substances [24]. From the test results in Table 2, it was found that the presence of aldehydes and ketones was not detected in the Sanping wine samples fermented with Hanseniaspora uvarum Hu2010 alone, but in the wines fermented with Saccharomyces cerevisiae D254 and Hanseniaspora uvarum Hu2010 for mixed bacterial fermentation The presence of 3,4-dimethylbenzaldehyde and damascenone was detected in the samples, which showed the aroma of rose and plum, pomelo, raspberry and other fruits, so in the sensory evaluation, the first group of wine samples and the second The floral and fruity aromas of the three groups of wines are more pronounced than those of the second group.

3.3. Sensory evaluation of wi

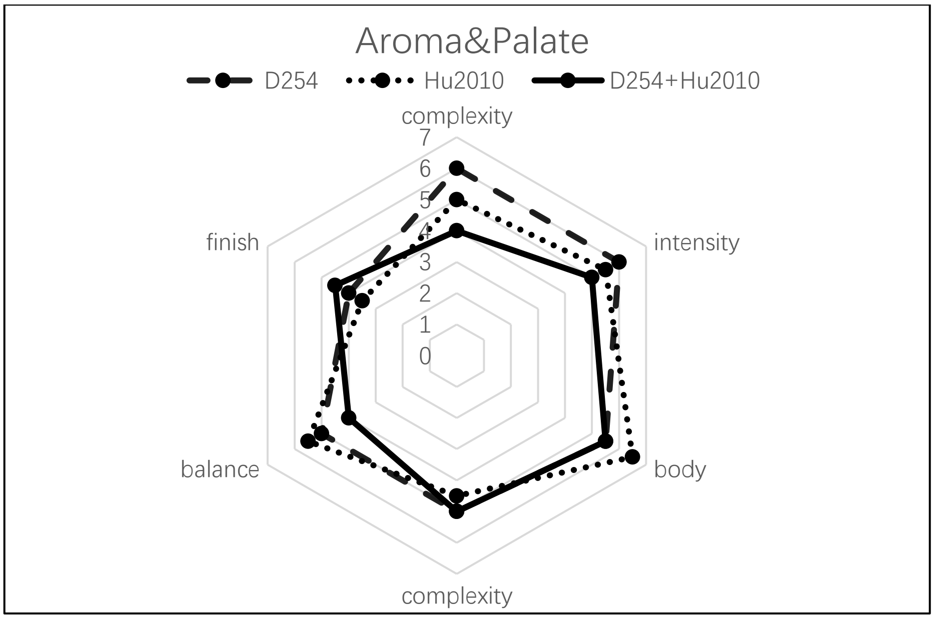

Figure 3. Sensory tasting of different yeast fermented wine samples

2.3.1 Aroma

As can be seen from Figure 3, from the perspective of olfactory complexity and intensity, compared with those inoculated with Saccharomyces cerevisiae D254, the two groups of wine samples inoculated with non-Saccharomyces cerevisiae have lower scores. Therefore, the use of non-Saccharomyces cerevisiae for separate fermentation Fermentation with mixed bacteria does not have much effect on the olfactory aroma of wine, and even reduces the quality of wine in olfactory aroma.

2.3.2 Palate

In terms of taste, all wine samples are similar in complexity of taste, generally a little thin, and there is no wine sample with outstanding layering; in terms of wine body, the second group was inoculated with Hanseniaspora uvarum Hu2010 wine alone This kind of wine is fuller, and feels thicker after entering the mouth; the taste balance is relatively high, and there is no obvious sourness in acidity, sugar, astringency, etc., and it is better to enter; On average, the wine samples in the third group inoculated with Hu2010 have longer fragrance retention; therefore, only in terms of taste as a whole, the wine samples in the second group inoculated with Hu2010 alone have a long history in terms of body, complexity and balance. , In terms of fragrance time, it is a relatively good bottle of wine.

In addition, it can be seen from Figure 3 that the effect of inoculation of non-Saccharomyces cerevisiae on wine samples is biased. For example, Hanseniaspora uvarum Hu2010 in grape juice can improve the body and balance of wine. The mixed inoculation of D254 and Hu2010 can enhance the complexity and aroma retention time of wine. Therefore, further research is needed to find a non-Saccharomyces cerevisiae that can improve the quality of wine in an all-round way.

2.3.3 Aromatype

Figure 4. Sensory tasting of different yeast fermented wine samples

Fig. 4 stipulates five kinds of tastes from the taste, respectively: floral, fruity, buttery, vanilla and esters. It can be seen that the D254 single fermented wine sample has a pleasant fruit aroma, and the vanilla and esters are obvious. The buttery and floral aromas are slightly lighter but the overall aroma is more balanced. The Hu2010+D254 fermented wine has the strongest floral and fruity aroma, obvious ester flavor, and insufficient vanilla and butter flavor; Hu2010 wine has the most prominent flower and fruit flavor, and the butter flavor is rich in ester flavor; but the overall aroma is insufficient. D254 wine sample has the most obvious ester flavor and the highest intensity. The two groups of mixed fermentation with non-Saccharomyces cerevisiae weakened the prominent ester flavor during single fermentation, and the flower and fruit flavor was prominent. In general, the two groups of wine samples fermented with mixed fermentation have higher floral aroma than Saccharomyces cerevisiae alone, and the fruit aromas of the three groups are almost similar. This may be because the content of alcohol esters metabolized by non-Saccharomyces cerevisiae is higher. Most of the esters have a certain amount of flowers and fruits, which can significantly enhance the pleasant aroma of flowers and fruits in wine, butter flavor, etc., and the aroma is relatively balanced. The wine sample fermented by Saccharomyces cerevisiae D254 alone is more complex in olfactory sense, but slightly inferior to the other two groups of wine samples in terms of taste balance, so the sourness is more obvious at the entrance, and its floral aroma is not strong , but with a strong vanilla and ester flavor. The wine samples fermented by non-Saccharomyces cerevisiae Hu2010 alone have a relatively pure aroma on the whole, with a fuller body, a strong floral and fruity aroma, a strong ester flavor, and a higher aroma complexity and overall coordination. The mixed fermented wine of non-Saccharomyces cerevisiae Hu2010 and D254 has a high content of esters, so its flower and fruit aroma is more obvious, but the wine body is relatively weak. When you enter the mouth, you can clearly feel the sweetness of the wine sample is very high, and the aftertaste Has a slight dairy taste.

4. Conclusion

Through the analysis of physical and chemical indicators, it can be seen that compared with the addition of Saccharomyces cerevisiae wine samples alone, the addition of *Hanseniaspora uvarum* Hu2010 will have a certain impact on the total acid, total sugar and reducing sugar in the wine samples, but It has little effect on alcohol content.According to the aroma substances detected by GC-MS, the use of non-Saccharomyces cerevisiae will produce different and unique substances in wine. For example, the unique substances in the wine samples fermented with Hu2010 alone are ethyl n-hexanoate, which has fruit Aroma: The unique hexyl formate in mixed fermentation with Hu2010+D254 will have apple and unripe plum-like sweetness.From the results of sensory evaluation of wine samples, it can be seen that the use of non-Saccharomyces cerevisiae for single-bacteria and mixed-bacteria fermentation can enhance the complexity of the wine body and the time of fragrance retention to a certain extent, but it may also weaken the sense of smell of the wine samples. on the intensity.The results show that the use of non-Saccharomyces cerevisiae to ferment grape juice can improve the quality of wine to a certain extent, and Hanseniaspora uvarum Hu2010 alone is better than the use of mixed fermentation to improve the overall quality of wine, but it is not comprehensive. improve the quality of wine. Although the use of grape juice Hanseniaspora uvarum Hu2010 alone fermentation makes the wine more mellow and the taste is more balanced, it also reduces the fragrance retention time of the wine in the mouth, and the olfactory complexity and richness are also improved. Therefore, the use of non-Saccharomyces cerevisiae for separate fermentation has certain limitations on the quality of wine, and further research is needed before it can be applied to specific production practices.

References

[1] Ma Yue, Tang Ke, Xu Yan, et al. Analysis of Aroma-Active Compounds in Wydal Ice Wines by Solid Phase Extraction Combined with GC-O/MS [J]. Food and Fermentation Industry, 2015, 41 (4): 153-159.

[2] Zhao Xinjie, Zhang Jiarong, Qin Shaozhi, et al. Aroma composition analysis of rose ice wine [J]. Wine Science and Technology, 2013 (9): 107-111.

[3] ZHANG Lizhu. Isolation, screening and tolerability of yeast in Weddell ice grapes on the Sichuan-Tibet Plateau [J]. Brewing in China, 2013, 32 (10): 94-97.

[4] Li Hua, Wang Hua, Yuan Chunlong, et al. Wine technology [M]. Beijing: Science Press, 2007: 68 – 73, 165-178.

[5] WANG Xingchen, HU Kai, TAO Yongsheng. Kinetics of mixed alcoholic fermentation of henson's yeast and Saccharomyces cerevisiae in grape juice [J]. Food Science, 2016, 37 (3): 103-108.

[6] CHAROENCHAI C, FLEET G H, HENSCHKE P A, et al. Screening of non-Saccharomyces wine yeasts for the presence of extracellular hydrolytic enzymes [J]. Australian Journal of Grape and Wine Research, 1997, 3 (1): 2-8.

[7] PINA C, SANTOS C, COUTO J A, et al. Ethanol tolerance of five non-Saccharomyces wine yeasts in comparison with a strain of Saccharomyces cerevisiae—influence of different culture conditions [J]. Food Microbiol, 2004, 21 (4): 439-447.

[8] KAPSOPOULOU K, MOURTZINI A, ANTHOULAS M, et al. Biological acidification during grape must fermentation using mixed cultures of Kluyveromyces thermotolerans and Saccharomyces cerevisiae [J]. World Journal of Microbiology and Biotechnology, 2007, 23 (5): 735-739.

[9] CONTRERAS A, CURTIN C, VARELA C. Yeast population dynamics reveal a potential 'collaboration' between Metschnikowia pulcherrima and Saccharomyces uvarum for the production of reduced alcohol wines during Shiraz fermentation [J]. Appl Microbiol Biotechnol, 2015, 99: 1885-1895.

[10] WAN Zhibo, QIAO Dan, YANG Tao, DONG Rong, MU Jingyi, ZHANG Zhenzhen. Study on aroma quality characteristics of Small Munson wine in Xinjiang appellation [J]. China Brewing, 2020, 39 (09): 106-110.

[11] ZHANG Yu. Study of the changes in aromatic substances in Musales wine using electronic nose and GC-MS techniques [D]. Tarim University, 2017.

[12] Tao Yongsheng, Peng Chuantao. Correlation analysis of aroma characteristics and composition of Chardonnay dry white wine in China [J]. Transactions of the Chinese Society for Agricultural Machinery, 2012, 43 (03): 130-139.

[13] Yan Hejing, Zhang Mingyu, Sun Kang, Yang Xiaokuan, Ge Chao, Zhou Jiefang, Liu Chang, Zou Jing. Effects of mixed fermentation of wild Saccharomyces cerevisiae and grape juice With Henson's yeast on aroma of rosy wine [J]. Food and Fermentation Industry, 2020, 46 (11): 165-171.

[14] Eugenio Revilla, Jose-Maria Ryan, Guillermo Martin- Ortega. Comparison of several procedures used for the extraction of anthocyanins from red grapes [J]. Agric. Food Chem, 1998, 46 (11): 592-597.

[15] Liu Tao, Ma Long, Tu Niansheng, Advances in the Biological Role of Grapes [J]. Nature Journal, 2002, 24 (2): 81: 87.

[16] Rapp A. Aroma compounds of wine [M]. Food Research International, 1989, 1125: 23-227.

[17] Seung Joo Lee, Ann C. Noble. Characterization of odor-active compounds in Californian Chardonnay wines using GC-Olfactometry and GC-Mass spectrometry [J]. Agric Food Chem 2003, 51: 8036-8044.

[18] Spayd S E, Tarara J M, Mee D L. Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries [J]. America Journal of Enology and Viticulture2002, 53 (3): 171-182.

[19] Maria Pilar Marti, Montserrat Mestres, Cristina Sala. Solid phase microextraction and chromatography olfactometry analysis of successively diluted samples. A new approach of thearoma extract dilution analysis applied to the characterization of wine aroma [J]. Agric Food Chem2003, 51: 7861-7865.

[20] Serkan Selli, Turgut Cabaroglu. Effect of contact on the aroma composition of the musts of Vitis vinifera Lcv. Muscat of Bormova and Narince grown in Turkey [J]. Food chemistry, 2003 (81): 341-347.

[21] Gholami M. Biosynthesis of flavour compounds in Muscat Cordor Blanc grape berries. Australian Journal of Grape and Wine Research, 1995, 1: 19-24.

[22] Liu Liyuan, Liu Yanlin, Li Hua. Research Progress on Wine Aroma Chemistry [J]. Food Science, 2011, 32 (05): 310-316.

[23] LI Hua. Aromatic substances of grapes[J]. Chinese and foreign grapes and wines, 2001 (06): 43-44.

[24] LI Hua, WANG Hua, LIU Laping, WANG Zhenqiang, SHUYing. GC/MS analysis of aroma components of Agri white wine [J]. Chinese Journal of Agricultural Sciences, 2005 (06): 1250-1254.