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Study on Changes of Chemical Components and Antioxidant Activity of Cherry Wine during Fermentation

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Using cherry as raw material, the anti-oxidation of cherry wine with different alcoholicity, vitamin C and time was determined and analyzed before and after the fermentation process, and the changes of antioxidant capacity in cherry juice before and after fermentation and during fermentation. The results showed that with the increase of alcoholicity, the content of flavonoids and polyphenols in cherry wine decreased gradually. When the alcoholicity reached 11%, the content of flavonoids and polyphenols was higher and the ability of scavenging hydroxyl radical was the best. The content of polyphenols and flavonoids was stable and the antioxidant capacity was the best. The content of polyphenols and flavonoids and the ability of scavenging hydroxyl radical were the best when 110 mg / L vitamin C was added. The fermentation time was 60h, 72h, 84h, Oxidation ability is better.

1. Introduction

Cherry tree is a kind of deciduous fruit tree belonging to the Rosaceae family, known as the first fruit in the early spring. When ripening, cherries are red, exquisite, delicious and nutritious. Cherry wine has far better anti-oxidation effect than other alcoholic drinks and can effectively enhance the immune system and reduce the risk of cancers and cardiovascular diseases. Our research object is its hydroxyl radical scavenging ability (Xiao et al., 2012; Sipos et al., 2017; Martinez et al., 2012). Although some researchers have optimized the fermentation process of cherry wine, none of these studies have addressed the effects of parameter adjustment on the content of bioactive components and their activity during fermentation. Therefore, this paper focuses on studying the fermentation conditions (such as fermentation temperature, inoculum size and amount of sugar added) on bioactive components and their biological activity, hoping to provide technical support for the further development of rich cherry resources (Liu et al., 2017).

2. Experiments

2.1 Materials and reagents

Materials: high-quality cherries and white sugar
Reagents: sodium hydroxide, sodium nitrite, aluminium nitrate, rutin, phosphoric acid, sodium tungstate, sodium molybdate, lithium sulphate, distilled water, hydrochloric acid, bromine water, anhydrous sodium carbonate, ethanol, phenanthroline, vitamin C, ferrous sulfate liquid, phosphate buffer, H2O2YJ-875 medical clean bench, bed temperature incubator, and benchtop drying oven from Chongqing Medical Equipment Factory; 751 GW UV, visible spectrophotometer from Hewlett-Packard Shanghai Analytical Instruments Co., Ltd.; JA2003 electronic balance from Shanghai Balance Instrument Factory; LD5-2A centrifuge from Beijing Medical Centrifuge Factory; microplate reader from Bio-Rad; GC/MS system GC20 from Shimadzu, used in conjunction with GC-Solution 7.0 data analysis workstation (Banović et al., 2008).
Instruments: UV765 UV spectrophotometer, FA1004B-type electronic balance and PHSJ-3F laboratory pH meter from Shanghai Precision Instrument Co., Ltd; 800 centrifuge from Shanghai Surgical Instrument Factory; GZC-9140MBE electric blast oven from Shanghai Boxun Industrial Co.; DL-5 low-speed high-capacity centrifuge from Shanghai Anting Scientific Instrument Factory; and FSH-2 multi-functional homogenizer from Wuhan Light Industry Company.

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2.2 Test preparations

2.2.1 Cherry wine fermentation process

Cherry → picking → cleaning → enucleating → crushing → filtration → adjusting components → inoculation → thermostatic fermentation → analysis and determination

2.2.2 Determination of indices

pH measurement: by acidometer.
Determination of alcohol volume fraction: alcohol volume fractions of cherry wine samples to be determined by alcoholmeter.
In order to study the impacts of different alcoholicity on the anti-oxidation effect of cherry wine, the author took 5 samples of cherry juice, each with a volume of 100ml, numbered as Sample 1, 2, 3, 4 and 5, respectively. The initial sugar content of the cherry juice was measured to be 77g/L. Based on the rule that 1.7g of sugar forms 1% alcohol, the author adjusted the sugar content by adding 0.8g, 4.2g, 7.6g, 11.0g and 14.4g, respectively. At a inoculum size of 5%, 5ml was inoculated with yeast 1596 and the samples were fermented at normal temperature for 7 days, making the alcoholicity reach 5%, 7%, 9%, 11% and 13%, respectively. Then the author studied the anti-oxidation effect of cherry wine by measuring its content of polyphenols and flavonoids and hydroxyl radical scavenging ability (Feng, 2008).
In order to study the anti-oxidation effect of cherry wine in different fermentation stages, the author took 500mL of cherry juice and added 63.5g of sugar. The SO2 concentration in cherry wine was adjusted to 60mg/L. Yeast 1596 was inoculated at a 5% inoculum size. From Day 1 to Day 7 during the fermentation, a sample was taken every 12h (Mitić et al., 2010; Milošević et al., 2016). By determining the content of polyphenols and flavonoids and the hydroxyl radical scavenging ability, this paper tries to study the anti-oxidation effect of cherry wine during fermentation.
Determination of free amino acid: the OPA method: the author accurately weighed 40mg of standard ortho-phthalaldehyde (OPA) and dissolved it in 1mL of methanol, added 2.5mL of 20% SDS solution, added 25mL of 0.1mol/L sodium tetraborate, 100μL of β-mercaptoethanol and diluted it with distilled water to 50mL (used right after it is ready). The author took 0mL, 0.04mL, 0.08mL, 0.12mL, 0.16mL and 0.20mL of standard amino acid solutions, added 0.20mL, 0.16mL, 0.12mL, 0.08mL, 0.04mL and 0.00mL of distilled water, and then added 4mL of OPA solution and shook it well. After letting it stand for 3min, the author measured the absorbance value where the wavelength was 340nm. With the amino acid concentration as the x-axis and the absorbance value as the y-axis, the author drew the curve and calculated the regression equation: y=0.0122x-0.0146 and the correlation coefficient R2=0.9978. Determination of free amino acids in the fermented broth: the author transferred 0.1mL of fermented supernatant, added 0.1mL of distilled water, shook it, added 4mLof OPA reagent and shook it well. After letting it stand for 3min, the author measured A340nm (Sun et al., 2016).

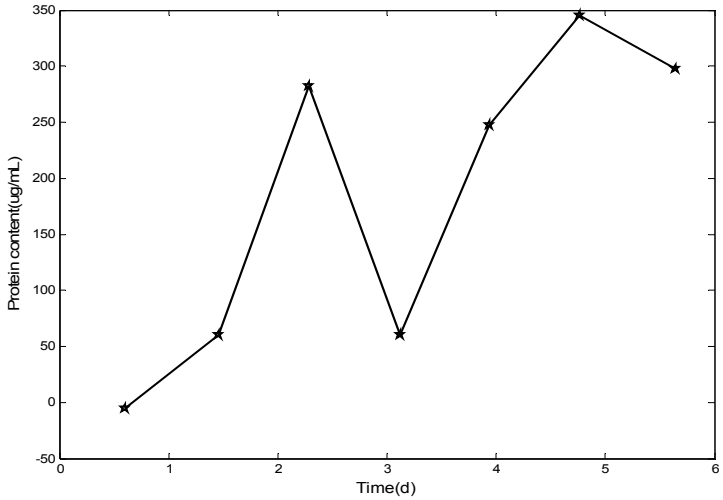

Figure 1: Changes of protein content during the fermentation

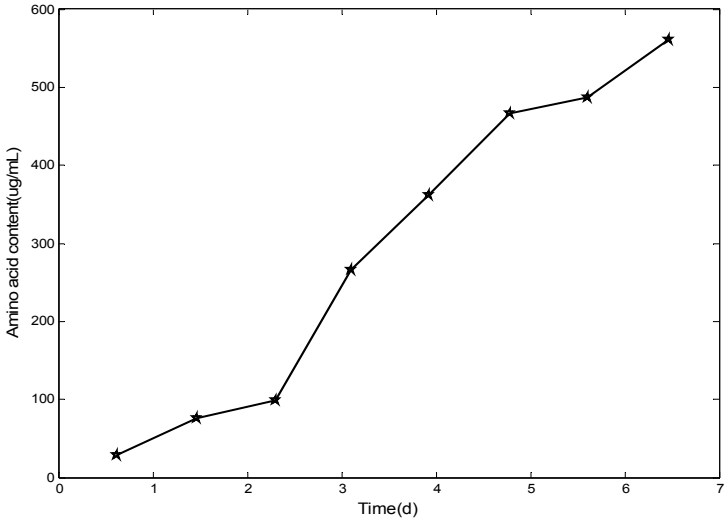

Figure 2: Changes of free amino acid content during the fermentation

2.3 Test method

ABTS free radical scavenging effect of the fermented broth: the author prepared 7mmol/L ABTS stock solution with 2.45mmol/L of potassium persulfate, diluted it with 10mmol/L phosphate buffer (pH7.4) so that the absorbance was 0.7+0.02 at a wavelength of 734nm, and then took 4mL of ATBS test solution and added 60μL of corresponding sample extract. After 6min of reaction, the author measured at A732nm and used it as A control. The author also took 4mL of ATBS test solution and added 60μL of sample solution. After 6min of reaction, the author measured at A732nm, which was regarded as A sample (Rios et al., 2010).

Scavenging rate =[(Acontrol-Asample)/Acontrol] ×100%

Reduction effect of the fermented broth in the FRAP system reduction: the author mixed 1mL of distilled water, 1.8mL of TPTZ solution and 60μL of sample solution, let it react at 37□ for 10min and then measured at A593nm. The result is expressed as Trolox equivalent anti-oxidation capacity (TEAC) in μmol/L.

3. Method for the anti-oxidation experiment

3.1 Changes in pH and alcoholicity

Fermented broths were taken at different fermentation stages for determination of acidity and alcoholicity. The changes of pH value and alcoholicity over the fermentation time are shown in Fig.3.

As can be seen from Fig.3, the pH value of the fermented broth decreased slightly in the early stage of fermentation. However, it began to rise back on Day 2, and finally stabilized between pH3.8-4.0. In addition, with the fermentation process, the volume fraction of the fruit wine continued to increase, especially on Day 2, when the alcohol volume fraction increased significantly. Afterwards, it began to rise slightly, mainly because the yeast used sugar to produce ethanol.

The total sugar content in the fermented broth of cherry wine decreased gradually, especially on Day 1, when the total sugar content dropped significantly, corresponding to the increase of the alcohol content in the broth. As can be seen from Fig.3, as the polysaccharide content in the fermented broth decreased, the alcoholicity of the fermented broth gradually increased, with a logarithmic relationship between the two.

3.2 Determination of indices

Flavonoids and polyphenols share a similar trend - both reached the highest content after Day 1 and then decreased slightly, but the content of flavonoids increased after 4 days and 7 days of fermentation, probably because most of the flavonoids and polyphenols in cherries are alcohol-soluble. With the fermentation time, the volume fraction of alcohol increased and the leaching amounts of flavonoids and polyphenols gradually increased, and in addition, the fermentation of cherries was carried out by shake-flask fermentation, which might lead to the oxidation of flavonoids and polyphenols leaching from the fermented broth. As a result, in the late stage, both the two experienced a slight decline in the content.

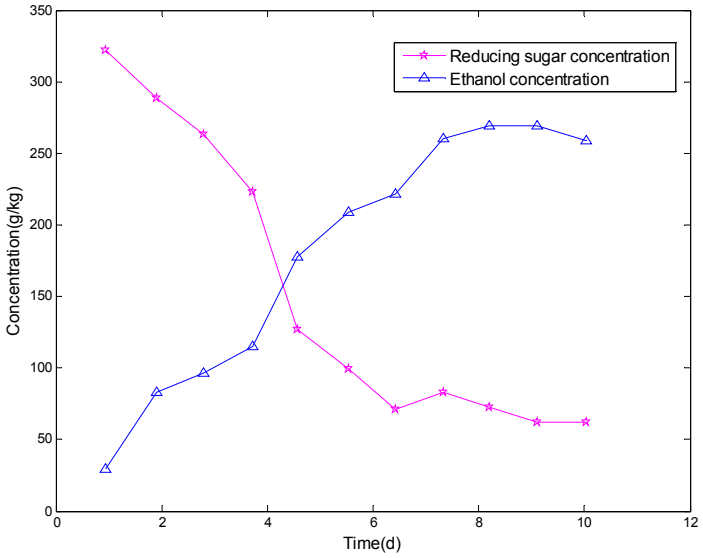

Figure 3: Ethanol production and sugar consumption during fermentation

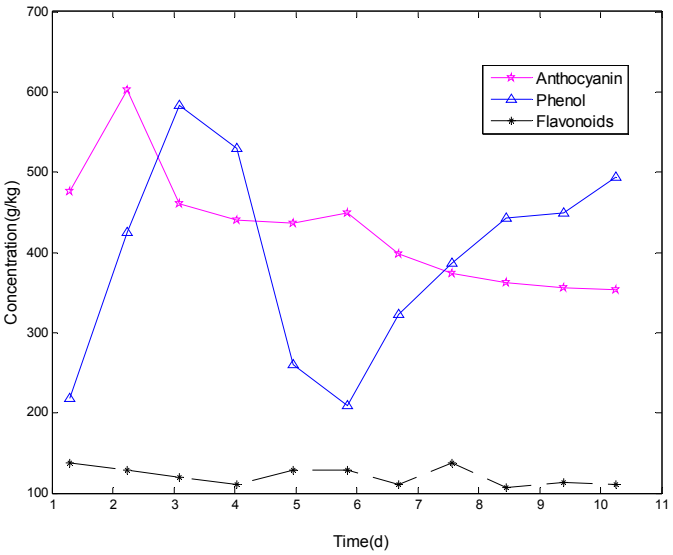

Figure 4: Flavonoids, anthocyanins andtotal phenol content change of mulberry wine during fermentation

3.3 Test on the impact of alcoholicity on the anti-oxidation effect of cherry wine

After 7 days of fermentation at room temperature, the author measured the absorbance values of flavonoids and polyphenols in the cherry wine with different alcoholicity and the AS for measuring the scavenging ability of hydroxyl free radicals (HO·). The results of the contents of flavonoids and polyphenols in cherry wine and the HO free radical scavenging rate (d) are shown in Table 1.

During fermentation, both flavonoids and polyphenols have the ability to scavenge free radicals to some extent. This anti-oxidation capacity has something to do with the hydrogen or electron donating ability of the phenolic hydroxyl, and the reaction is: Fl(OH)+·OH→Fl(O·) +H2O. In addition, the metal ions inhibit its catalytic action on oxygen. The difference in the anti-oxidation activity is closely related to the structure-activity relationship. From Table 3, it can be seen that with the alcoholicity increasing, the contents of flavonoids and polyphenols increased gradually and the hydroxyl radical scavenging ability also increased slowly. When the alcohol content reached 11%, the anti-oxidation capacity increased the most. The reason is that the molecular

structure of flavonoids promotes the electron delocalization and helps form a relatively stable radical intermediate after the hydrogen donor, thereby enhancing its anti-oxidation capacity. The HO· free radicals scavenging ability of polyphenols is shown in that after the reaction with ·OH, O replaced HO·, and the anti-oxidation capacity of polyphenols is closely related to its chemical structure – it is enhanced with the increase of polymerization degree. When the content of alcohol reached 13%, the contents of flavonoids and polyphenols decreased most rapidly - the former decreased by 14.1% and the latter by 15.1%, and the free radicals scavenging ability also increased by 36.7%. The reason for this is that different substitution positions of glycoside led to differences in the oxidation activity and the introduction of glycoside radicals reduced the anti-oxidation capacity. The anti-oxidation capacity of polyphenols also decreased with the decreasing polymerization degree. Therefore, the alcohol content should be controlled below 11%vol during the fermentation of cherry wine.

Table 1: Effect of Alcohol Content on Flavonoids, Polyphenols Content and HO Radical Scavenging Rate (d)

Alcohol content (%)	Flavonoids (μg·mL-1)	Polyphenol content (μg·mL-1)	HO·Free radical scavenging rate (%)
5	46.762	0.453	27.6
7	47.591	0.623	36.8
9	60.341	0.672	52.0
11	55.774	0.565	39.9

3.4 Test on the impact of fermentation time on the anti-oxidation effect of cherry wine

From Day 1 to Day 7 during the fermentation, samples were taken every 12h to measure the absorbance values of flavonoids and polyphenols in the cherry wine with different alcoholicity and the AS for measuring the scavenging ability of hydroxyl free radicals (HO·). The calculated results of the contents of flavonoids and polyphenols in cherry wine and the HO free radical scavenging rate (d) are shown in Table 2.

Table 2: Content of flavonoids and polyphenols during the fermentation

Fermentation time (h)	Flavonoids (μg·mL-1)	Polyphenol content (μg·mL-1)	HO·Free radical scavenging rate (%)
0	75.157	1.282	19.1
12	64.426	0.995	28.3
24	138.010	1.158	29.6
36	245.320	1.104	75.0
48	303.574	1.221	80.3

Compared with those of the unfermented cherry juice, during the initial fermentation, the contents of flavonoids and polyphenols decreased first, and then over the fermentation time, the contents of flavonoids and polyphenols and the hydroxyl radicals scavenging ability gradually increased. When the fermentation time exceeded 84hr, the contents of flavonoids and polyphenols and the hydroxyl radicals scavenging ability generally showed a downward trend. The decreases in the contents of flavonoids and polyphenols in the fermentation process may be caused by the following reasons: polyphenols is an important factor that affects the flavour stability of the fruit wine. Polyphenols contain a large number of oxidizable groups, significantly affecting the O/R potential. Appropriate amount of polyphenols can delay the flavour aging. The anti-oxidation effect of polyphenols is shown in the following three forms: (1) acting with free radicals: (2) inhibiting lipid peroxidation; (3) chelating compounds with copper, iron and other metal ions. The free radical theory about wine flavour aging has been widely accepted. In this aging process, reactive oxygen species (ROS) play a key role. The free radical reaction mechanism leads to the production of carbonyl compounds, which is considered as the main source of flavour aging. However, the anti-oxidants such as polyphenols and flavonoids can effectively inhibit the production of carbonyl compounds.

4. Conclusion

Antioxidant capacity of cherry fruit wine with the increase of alcohol content, the contents of flavonoids and polyphenols increased gradually, and their capacity of scavenging hydroxyl radicals increased slowly. When the alcohol content reached 11%, the antioxidant capacity increased most. When the cherry fruit in the

fermentation process, the yeast fermentation, most of the sugar into alcohol; flavonoids, polyphenols, although alcohol-soluble, but because cherry alcohol wine fermentation broth is not very high, and because of its easy oxidation, In the fermentation process its content decreased slowly after one day of fermentation until it remained unchanged; through the experimental results of protein and free amino acids, we can see that some of the protein is decomposed into free amino acids.

Acknowledgments

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