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Screening of Natural Antioxidants and Application In the Course of Perry Brewing

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Abstract

DPPH assay and FRAP assay (Ferric Reducing Antioxidant Power) were used to determine the antioxidant activity of forty kinds of Chinese herbs. Among them, eight were found to possess strong antioxidant activity including Magnolia and Rhus chinensis Mill.. Eight Chinese herbs were used to perry brewing. Total polyphenols were determined by Folin-Ciocalciu method in order to measure their antioxidant activity. The results showed that Magnolia and Rhus chinensis Mill. had high antioxidant activities in eight Chinese herbs in perry.

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1.Introduction

During the course of the wine production process, the oxidation and browning affect seriously the wine quality. The wine producer was worried by this question. The traditional antioxidant is mainly the sulfur dioxide. But the sulfur dioxide affects health. This question was attracted attention of people^[1]. The laws of European Union and France and other areas require that the content of sulfur dioxide must be marked on the wine label (not more than 10mg per litre). Looking for a replacement method for sulfur dioxide in the wine, reducing the content of sulfur dioxide had become an inevitable trend^[2-4]. In addition, studying of Wanghua et al. show that the sulfur dioxide can not play the role of the antioxidant under the condition of the specified content. Finding the new and efficient natural antioxidants had become a pressing matter of the moment of the wine producers and researchers. At present, the method of replacing completely the sulfur dioxide has not be found. Some reports was still in the stage of

researching^[5-6]. But a large number of data analysis showed that the adding antioxidants is a economic and practical measure.^[7-10]

There are rich Chinese herbs resources in our country and many kinds of antioxidants in them. It is feasible to finding the wine antioxidants from Chinese herbs. Three methods of DPPH, FRAP and Folin phenol was used to research the antioxidant activity of forty kinds of Chinese herbs in the paper.

2. Material and methods

2.1 Material and reagents

Forty kinds of Chinese herbs were purchased from Hunan province Changsha city.

DPPH (1, 1-two phenyl-2-picryl hydrazine radical) Sigma corporation. L-methionine Sea Olsen company. Riboflavin and Folin Phenol reagent Shanghai Lida limited company of biology science and technology.

2.2 Methods

- *Crude extracting liquid preparation*

The 5g Chinese herbs were put into the mortar and crushed. Ten times distilled water was added to the above liquid and made the homogenate. It was extracted 10 minutes by the ultrasound and static 90 minutes.

- *DPPH method*

Ethanol (95%, v/v) solution of 1.95ml, 24mg/L DPPH was put into the cuvette and determined the absorbance under the 515nm wavelength. Then 50 μ l sample was added to the above solution. The absorbance value was regarded at 2, 3, 4, 5 minute. After 5 minutes the absorbance value was determined at each 5 minutes until the change of the absorbance value was no more than 0.003 units^[11].

- *FRAP method*

The 900 μ l FRAP solution of freshly prepared was put at 37°C and mixed with the 90 μ l double distilled water and 30 μ l sample. The absorbance value was determined under 593nm after reacted 10min at 37°C^[12].

FRAP solution: 2.5ml 10mmol/L TPTZ solution, 2.5ml 20mmol/L FeCl₃·6H₂O and 25ml 0.3mol/L acetate buffer solution, pH3.6^[13].

- *Determination of total phenolics content by the folin phenol method*

- *Method*

The pear wine with the antioxidants of 0.5ml were measured with a pipette precise to the 50ml volumetric flask. The 30ml distilled water was added to the flask and shook well. Then 2.5ml folin phenol and 7.5ml 20% Na₂CO₃ were added and heated to 75°C for 10minutes. After cooling the absorbance value was measured at 760nm wavelength^[14].

- *Building of standard curve*

The gallic acid of 0.1mg/ml was measured 0, 0.5, 1, 1.5, 2, 2.5, 3ml to the 50ml volumetric flask and added 30ml water.

3. Results and analysis

3.1 40 kinds of Chinese herbs extracts antioxidant results analysis

Eight kinds of Chinese herbs including of Magnolia, gallnut, Terminalia chebula meat, Amomum villosum, clove, liquorice, olives and Vitex showed strong antioxidant capacity. The DPPH removal rate reached above 90% (Table 1). Free radical scavenging ability sequence is Magnolia > gallnut > Terminalia chebula > meat of Amomum villosum > clove > Zhili grass > oregano > Vitex negundo

Table 1. Antioxidation effectiveness of Water Extracts of Chinese Herbs

Chinese herbs names	DPPH (IP%) \pm SD	FRAP (相当于 FeSO ₄ 的量) \pm SD
Magnolia officinalis	98.251 \pm 0.224	4.135 \pm 0.023
Gallnut	96.162 \pm 0.023	3.854 \pm 0.004
Terminalia chebula meat	95.629 \pm 0.057	3.576 \pm 0.062
Amomum villosum	94.247 \pm 0.103	2.519 \pm 0.007
Lilac	92.387 \pm 0.005	1.268 \pm 0.014
Licorice root	90.452 \pm 0.243	1.127 \pm 0.032
Olive	90.326 \pm 0.025	1.134 \pm 0.002
Vitex negundo	90.037 \pm 0.008	1.052 \pm 0.005
Dried tangerine peel	88.152 \pm 0.002	0.924 \pm 0.048
Ginseng	87.737 \pm 0.030	0.931 \pm 0.003
Acanthopanax senticosus	85.326 \pm 0.004	0.886 \pm 0.023
Black cohosh	85.218 \pm 0.027	0.873 \pm 0.002
Astragalus	84.867 \pm 0.043	0.857 \pm 0.008
Ophiopogon japonicus	83.754 \pm 0.022	0.824 \pm 0.043
Digitalis	83.229 \pm 0.003	0.837 \pm 0.027
Codonopsis pilosula	81.651 \pm 0.001	0.795 \pm 0.006
Asarum	80.015 \pm 0.025	0.763 \pm 0.001
Oregano	79.637 \pm 0.050	0.528 \pm 0.004
Spearmint	77.473 \pm 0.009	0.543 \pm 0.058
Lavender	75.284 \pm 0.018	0.537 \pm 0.009
Perilla frutescens	74.516 \pm 0.026	0.484 \pm 0.005
Perilla crispa	72.349 \pm 0.417	0.468 \pm 0.062
Zhili grass	72.349 \pm 0.417	0.468 \pm 0.062
Cherokee rose	70.251 \pm 0.015	0.465 \pm 0.051
Wu aconite	67.537 \pm 0.027	0.412 \pm 0.012
Golden buckwheat	66.215 \pm 0.023	0.364 \pm 0.007
Rhodiola rosea	63.154 \pm 0.035	0.375 \pm 0.005
Salvia	61.235 \pm 0.018	0.216 \pm 0.025
Sophora japonica	58.126 \pm 0.006	0.125 \pm 0.006
Eupatorium	46.348 \pm 0.012	0.106 \pm 0.014
Ligustrum lucidum	43.241 \pm 0.004	0.096 \pm 0.007
Chinese wolfberry	42.572 \pm 0.078	0.063 \pm 0.003
Students of	30.315 \pm 0.047	0.060 \pm 0.058

Polygonum multiflorum		
Three seven	27.426±0.004	0.057±0.062
Patchouli	24.605±0.036	0.035±0.004
Marjoram	22.324±0.008	0.027±0.002
Semen Cassiae	18.645±0.005	0.034±0.008
Scutellaria	12.182±0.036	0.026±0.027
Pepper	10.871±0.043	-0.002±0.024
Ginkgo	2.327±0.007	-0.042±0.004
Kelp	1.259±0.015	-0.053±0.001

In addition orange peel, ginseng, acanthopanax senticosus, Cimicifuga, astragalus, Codonopsis, Radix Ophiopogonis, digitalis, asarum DPPH clearance rate has reached more than 80%, have strong antioxidant ability. At the same time, using the FRAP method for the determination of the sample reduction capacity, in addition to an antioxidant capacity of different, the rest are consistent, two methods to get similar results.

As a result of Chinese traditional medicine most contain polyphenols, alkaloids, ketones and quinone chemical components from traditional Chinese medicine, the screening of antioxidants has great feasibility, combined with traditional Chinese medicine is rich in resources, the development of Chinese medicine antioxidants will become the hot spot of the development. On the basis of this, the researchers will be above 8 kinds of traditional Chinese medicine as the research object, looking for a more suitable for application to Perry natural antioxidants in wine, to replace sulfur dioxide antioxidant effect, thereby reducing the amount of sulfur dioxide.

3.2 Results of the total phenolics content by the folin phenol method

- *Building of standard curve*

Table 2. Gallic acid content and the corresponding absorbency values

Gallic acid content (µg/ml)	0	1	2	3	4	5	6
absorbance value(A _{760nm})	0.009	0.104	0.203	0.277	0.441	0.506	0.551

The standard curve as shown in figure 1.

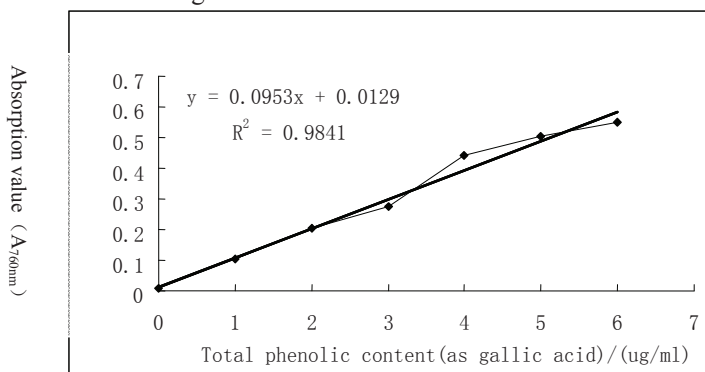


Figure 1. Standard curve of total polyphenol content determination

Data can be obtained by standard curve corresponding to the equations as follows:

$$x = \frac{y - 0.0129}{0.0953}$$

y absorbance value x total phenolic content ($\mu\text{g/ml}$)

- The total phenol content in pear wine with Chinese herbs as antioxidant

Table 3. Result of polyphenol content of perry with Chinese herbs

sample names	absorbance value ($A_{760\text{nm}}$)	total phenolic content (B, $\mu\text{g/ml}$)
Magnolia officinalis	0.375	3.800
Gallnut	0.360	3.642
Terminalia chebula meat	0.326	3.285
Amomum villosum	0.332	3.348
Lilac	0.298	2.992
Zhili grass	0.273	2.729
Oregano	0.304	3.055
Vitex negundo	0.156	1.502
Sulfur dioxide	0.359	3.632

Eight kinds of Chinese herbs with strong antioxidant effects were screened by using the DPPH and FRAP method. These Chinese herbs as antioxidants were added to the pear wine. Sulfur dioxide was regarded as control (Table 3). Magnolia and gallic have more stronger antioxidant activity than sulfur dioxide. The total phenolic content in pear wine with magnolia and gallic reached $3.800 \mu\text{g/ml}$ and $3.642 \mu\text{g/ml}$. The two kinds of Chinese herbs have the potential activity instead of sulfur dioxide.

4. Conclusion

The antioxidant capacity of forty kinds of Chinese herbs were determined through DPPH and FRAP. Eight kinds of them including Magnolia officinalis, Gallnut, Terminalia chebula meat, Amomum villosum, Lilac, Zhili grass, Oregano and Vitex negundo have strong antioxidant ability by above methods. The eight kinds of Chinese herbs were applied to the pear wine brewing process. Magnolia officinalis and gallic were founded to have still strong antioxidant activity. They has the potential to replace sulfur dioxide.

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