

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.503.004>

Evaluation of Antioxidant and Antimicrobial Activity of Wine from Various Sources

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ABSTRACT

Keywords

Wine, Antioxidant property, Antimicrobial activity, Strawberry, Barley, Rice.

Article Info

Accepted:
07 February 2016
Available Online:
10, March 2016

The aim of present study is to produce wine from four different sources- rice, barley, grapes and strawberry. The wine produced is determined for its antioxidant properties, total phenolic compounds and antimicrobial activity. The quantification and purification of the wine samples was carried through HPLC. The antioxidant and the efficacy of wine against various microorganisms was verified through disc diffusion method. The assay was performed for four consecutive weeks after the production of wine, and the comparative analysis was made. All the observations suggest that wines have potential effect to overcome the oxidative stress and prevent cellular damage, which is a root cause of various forms of cancer and heart diseases.

Introduction

Wine rich in antioxidant property an alcoholic beverage also is considered as an excellent source for many bioactive compounds like antioxidants, polyphenolic and flavonoids. Reports suggest that wine contains antioxidant power that reduces the oxidative stress and prevents risk for many coronary diseases and cancer (Danila Di Majo *et al.*, 2008). The respiratory cycle of oxidative phosphorylation produces free radicals which may attack biological macromolecules like DNA, and cause cell ageing, cardiovascular diseases, and even cancerous tumor growth due to denaturation of single and double strand DNA.

When immune system of an organism fails to rectify the problem then the situation of 'oxidative stress' occurs, which damages the macromolecules present in the body. Consumption of food containing natural antioxidant activity is one of the effective way to overcome the problem (Reşat Apak *et al.*, 2007; Just J.R. *et al.*, 2003; Daroch *et al.*, 2001). Besides these properties, wine also shows significant antimicrobial activity. Recently, the antimicrobial effects on various plant extracts against certain pathogens like *Staphylococcus aureus*, *Candida albicans* and *E.coli* have been reported by a number of researchers. The

polyphenols like resveratrol, hydroxytyrosol, quercetin and a number of phenolic acids present in the wine shows anti-oxidant, anti-carcinogenic, anti-inflammatory and anti-microbial activity (Chrissanthy Papadopoulou *et al.*, 2005; Sandipan Goswami and Subhajit Ray, 2010; Bapat, 2010).

Wine is produced from wide variety of sources. For the winemaker's continuous production of wine throughout the season, its cost effectiveness, easy transportation, storage are essential factors. The quality of the fruit, amount of fermentable sugars, acid content and characteristics flavor provides suitability for wine making (Sandipan Goswami and Subhajit Ray, 2010). Most of the winemaker prefers grape for wine production. The total phenolics in red wine are higher in comparison to the white wine. Wine phenolics are categorized as flavonoid and non-flavonoids. Few fruits with balanced sugar, acid, tannin, nutritive salts as a suitable substrate for the growth of yeast with adequate amount of water are also used for wine production (Robinson Jancis, 2006). The Arunachal Pradesh Horticulture Produce Marketing & Processing Board (APHM&PM) in India have funded the project for the wine processing from kiwi fruit. This fruit has high aroma and a refreshing flavor with high acidic nature and sugar almost similar to grapes (Business Standards, 2015). Sucrose if generally added for the fruits that have excess acids can split into fermentable fructose and glucose. In order to maintain the specific gravity of the juice at the initial is too high with water or acidulated water it can be adjusted to the winemaker's target range (Bapat *et al.*, 2010).

In this present study wine was prepared from four different sources; rice, barley, grapes and strawberry under different

fermentable conditions. The characterization of both red and white wine was carried out and compared with the commercially available wine for comparative analysis. The quantification and purification of the wine was carried out by HPLC. A comparative evaluation was performed for the estimation of total antioxidant compounds, phenolics concentration and its antimicrobial activity.

Materials and Methods

Production of Wine from Different Sources

Processing and Fermentation

Production of Wine from Black Grapes (*Vitis vinifera*)

250g of grapes was weighed and washed by removing the stems with 1% HgCl₂ then with sterile distilled water for 10 minutes. The grapes was soaked in 5% sodium chloride solution for 15minutes. After the completion of surface sterilization finally it was washed with sterile sterile distilled water. The inoculum was prepared for batch fermentation by homogenizing 250g of grapes with 25g of sugar and 200ml of sterile water into an pre-autoclaved wine jar fitted with rubber cork and bent glass tube for the release of CO₂. The yeast cell was cultured and maintained for 48 hours in yeast extract, peptone dextrose medium at 27⁰ C. The inoculum size of 3 x 10⁴ cells /mL was transferred into the fermentation wine jar containing grape juice. The inoculum bottle was incubated for 48 hours at room temperature under slow shaking condition. Later 750g of grapes was homogenised along with 50g of sugar and 300ml of water. A pinch of potassium metabisulphite was added. The inoculum was transferred completely to this fermentation bottle and allowed for

production of wine. The contents are mixed well and incubated for 28 days at room temperature under stationary condition during the incubation period. After the completion of the incubation period of 28 days quantification of antioxidants, total phenolic compounds and other characterization of wine was performed for every week until 4 weeks.

Production of Wine from Strawberries (*Fragaria ananassa*)

The strawberries was cleaned by removing the stems and washed under tap water. 250g strawberries are then firstly washed with 1 % HgCl₂ then with sterile distilled water. The cleaned fruits was soaked in 5% sodium chloride solution for 15 minutes washed with sterile distilled water. The strawberries was then weighed and homogenised into pulp in a blender along with 25g of sugar and 200ml of water and transferred into a brown bottle. The same concentration of yeast culture was added as a starter culture to the fruit extract and incubated at 27⁰C for 48 hours under slow shaking condition. For the batch fermentation process same set up was made for the wine production as in the case of grapes. The remaining 750g of strawberries are then homogenized into pulp along with 50g of sugar and 300ml of water. A pinch of potassium metabisulphite is added to it and the inoculum is transferred. The contents are mixed well and incubated for 28 days (Sandipan Goswami and Subhajit Ray, 2010) under stationary condition.

Production of Wine from Rice (*Oryza sativa*) and Barley (*Hordeum vulgariae*)

The wine production from rice and barley was performed under two conditions: With sugar and without sugar as the sugar content in both are comparatively lesser than the

fruits.

Production of Wine from Rice (Half Boiled Rice)

The rice grains was cleaned and washed with water properly. It was soaked in clean sterilized water for an overnight and was grind to small particles. In the first setup: 500g of partially grinded rice in a 1liter wine jar with single holed rubber cork with a bent glass fitted to release the CO₂ during the fermentation and 200ml of water is taken. To this 17g of sugar and same inoculums size of precultured yeast added to the jar. In the second setup: Another 500g of grinded rice was taken similar to the previous set without adding sugar. A pinch of potassium metabisulphite was added to both the jars and the contents were mixed well and incubated for 48 hours under slow shaking condition then transferred to stationary condition for 26 days of incubation.

Production of Wine from Barley

The barley was cleaned and washed under tap water. It was soaked in clean sterilized water for an overnight and was grind to small particles. Two experimental set up was established-with sugar and without sugar. In the first set 500g of partially grinded barley was taken in a 1liter wine jar with 22g of sugar and 200ml of water. The rest 500g of grinded barley was set for wine production without sugar. An yeast inoculum of 3 x10⁴ cells/mL was added to both the wine jars and incubated for slow shaking condition for initial 48 hours and later maintained the fermentation process under stationary condition for 02 days. A pinch of potassium metabisulphite is added to both the jars and the contents were mixed well and incubated for 28 days (Sandipan Goswami and Subhajit Ray, 2010).

Characterization of Standard Wine and Prepared Wine

Estimation of Percentage of Alcohol, Total Acid and Volatile Acid

Estimation of alcohol The concentration of alcohol was estimated by alcoholmetry method using AOAC chart (Table 1).

Estimation of Total Acidity and Volatile Acidity

10ml of the standard wine as well as sample wines was taken in a 100ml conical flask. Diluted to 1:1 ratio by adding 10ml of distilled water and a few drops of phenolphthalein indicator. This solution was titrated against 0.1N NaOH for the colour appearance from pink to green. The titration was repeated to get concordant values (Ronald *et al.*, 2010). The total acidity and volatile acidity was estimated from the titrated value (Table 1).

Purification and Quantification of Bioactives Compounds of Wine Samples by HPLC

The column used for HPLC system consisted of a C18 RS column (250, 4.6mm ID, S-5 μ m, 8nm) separation was performed at room temperature. Injection volume was 10 μ L, flow rate set at 1 mL/min with UV detector was carried out at 290 nm. Solvent used were 2% acetic acid and 0.5% acetic acid / 50% acetonitrile. 1 mL of wine sample was diluted with 5mL of methanol and filtered through 0.45 μ m filter before injection into the HPLC⁴ (Table 2).

Estimation of Antioxidants Present in Different Wine Samples Phosphomolybdenum Assay Protocol

An aliquot of 0.3 ml wine sample (5 mM in MeOH) was combined in a 4-ml vial with 3

ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials was capped and incubated in a water bath at 95°C for 90mins. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695nm against a blank. The antioxidant activity was (Kashif Ghafoor *et al.*, 2011) expressed relative to that of ascorbic acid (Kashif Ghafoor *et al.*, 2011) Table 3.

Antimicrobial Assay (Disc Diffusion Method)

The pure bacterial and fungal culture was inoculated in a freshly prepared nutrient broth and potato dextrose broth respectively. The inoculated broth was incubated until the OD at 620nm reaches 0.7. The cultures from the broth was swabbed on a Mueller Hinton agar plate for bacteria and potato dextrose agar plate for fungal culture. The discs with wine samples of various concentrations was placed on the petri plates with swabbed cultures using sterile forceps. The plates was incubated for 24hrs for bacterial culture and 3 days for fungal culture. After the completion of incubation period the zone of inhibition was measured and compared with standards (Zahid Hussain, 2011) Kirby Bauer Chart The antimicrobial activity shown by different wine samples for microbial cultures with the zone of inhibition was compared for different wine samples (Table 4).

Estimation of Total Phenolic Compounds

The total phenolic compounds was analyzed using Folin Ciocalteu method. A 200 μ L properly diluted wine sample or standard solution of varying concentrations mixed with 400 μ L Folin Ciocalteu reagent. The deionized water was used for dilution and control. The solution was diluted to a total volume of 4.6 mL using deionized water

followed by thorough mixing. After incubation for 10 min at room temperature, 1 mL of 10% Na₂CO₃ solution was added followed by immediate thorough mixing and incubated for 2hrs at room temperature. The absorbance was read at 765nm on a spectrophotometer (Kashif Ghafoor *et al.*, 2011). Gallic acid of 1 mg/mL was used as the standard and the total phenolic compounds of the samples was expressed in milligram gallic acid equivalent per mL of wine sample (mg GA/1 mL) (Table 5).

Results and Discussion

Characterization of Prepared Wine Samples

The prepared wine samples was characterized by the colour, aroma, flavor, astringency, pH, alcohol percentage, total acid and volatile acid (Table1). The observations after the 3rd till 9th week fermented wines was found as grape wine was light reddish purple color, strawberry wine was pinkish, rice and barley wine (with and without sugar) was creamish white. The pH of grape and strawberry wine was found to be 4.7 after fermentation whereas pH was 3 before fermentation. Rice and barley wine (with and without sugar) the pH was found to be 4.0 after fermentation and pH 6 before fermentation (Table1).

The alcohol concentration ethyl alcohol, propanol, butanol, etc) was estimated by specific gravity method using specific gravity bottle. The specific gravity of all the wine samples was calculated and percentage of alcohol present was estimated using AOAC chart (Table 1).

Total acid in this study was expressed in terms of percentage of Tartaric acid (Table 1) and was calculated by the formula: Total acidity (TA) =Volume of NaOH added x Normality of NaOH x 7.5/10.

Volatile acid is expressed in terms of percentage of Acetic acid (Table 1) and is calculated by the formula: Volatile acidity (VA) = Volume of NaOH added x Normality of NaOH x 6/10.

Total acid and volatile acid directly effects the color and flavor of wine. Tartaric acid is one of the major acidic material present predominantly in compare to other acids like malic acid, acetic acid etc. and helps in the ageing of the wine. At the same time among the various volatile acids present in the wine acetic acid plays the major role (Wahab *et al.*, 2005).

The tartaric acid in Roselle wine was found to be 0.65% which is almost close to grape wine in the present study (Arubi *et al.*, 2009). The VA of the Roselle wine showed 0.04% of acetic acid, where the rice wine prepared with sugar in the present study showed 0.035% of acetic acid (Table 1). It is observed that grape wine has highest percentage of TA and VA, followed by strawberry, rice (with sugar), rice (without sugar), barley (with sugar) and lastly barley (without sugar).

Reddy *et al.*, in 2005 (Reddy *et al.*, 2005) optimised wine production from six varieties of *Mangifer indica* L and it was found that ethanol produced was 7-8.5%, TA (tartaric acid) 0.6-0.8 % and VA (acetic acid) was 0.01-0.2% v/v.

In 2009 Soni *et al.*²¹ reported during the batch process alcohol produced was 12% with supplementation of ammonium sulphate, potassium dihydrogen phosphate, proline and biotin to hot water extract of *Emblica officinalis* (Indian gooseberry), but later in a fed batch process the concentration of alcohol was increased to 16.1 %.

The formation of various alcohols like ethyl alcohol, n-propanol, n-butanol, isobutanol

and isoamyl alcohol were formed 9% v/v, 33.45, 8.9, 58.98 & 120.67 mg/L respectively.

Identification of Bioactives and Purification of Prepared Wine Samples by High Performance Liquid Chromatography (HPLC)

The HPLC was performed for all the prepared wine samples in the 5th week of fermentation and the amount of antioxidant present in each sample is calculated, and is expressed in $\mu\text{g/mL}$. Gallic acid was used as a standard (Table 2).

The result represents that the concentration of antioxidants presents in strawberry was highest $20\mu\text{g/mL}$. and for grapes $12\mu\text{g/mL}$. and barley with sugar $10\mu\text{g/mL}$. The other samples contained lower concentration of antioxidant.

Estimation of Antioxidants Present in Different Wine Samples by Phosphomolybdenum Assay

The assay was conducted for four consecutive weeks for comparative analysis (Table 3). The amount of antioxidants present in each wine sample was estimated and expressed in terms of $\mu\text{g/mL}$. Ascorbic acid was used as standard. It was observed that the amount of antioxidants in all the wine samples increases as the weeks for fermentation increase. Strawberry wine showed constant increase in the concentration from $60\mu\text{g/mL}$ to $100\mu\text{g/mL}$. Grape wine, rice with sugar and barley with sugar were also showed presence of antioxidant very comparatively close to each other. Hence, in the upcoming days huge interest could be shown in the cultivation of strawberries and the production of strawberry wine.

Table.1 Characterization of Prepared Wine Samples After 28 days of Incubation

Wine samples	Colour	Aroma	Astringency	pH	Alcohol %	Total Acid%	Volatile Acid %
Grapes	Reddish Purple	Sour Fruity	Moderate	5	14	0.71	0.056
Strawberries	Pinkish	Sweet Fruity	Moderate	5	13	0.58	0.052
Rice (with sugar)	Creamish	Fermented food	High	4	13	0.27	0.039
Rice (without sugar)	Creamish	Fermented food	High	4	11.7	0.19	0.033
Barley (with sugar)	Creamish	Fermented food	High	4	12.5	0.35	0.038
Barley (without sugar)	Creamish	Fermented food	high	4	11.5	0.27	0.024

Table.2 Amount of Antioxidants (Mg/MI) Estimated After 28 Days in Different Wine Samples

Wine samples	Amount of antioxidant($\mu\text{g/ml}$)
Grapes	19
Strawberries	26
Rice (with sugar)	8
Rice (without sugar)	5
Barley (with sugar)	15
Barley (without sugar)	6

Table.3 Estimation of Antioxidants from Different Wine After the Fermentation (28 Days) Till Four Weeks

Wine samples	6th week	7 th week	8 th week	9 th week
Rice (with sugar)	12	26	32	40
Rice (without sugar)	8	10	18	26
Barley (with sugar)	22	40	46	52
Barley (without sugar)	18	20	23	30
Grapes	18	28	52	60
Strawberry	60	66	80	100

Table.4 Study of Antimicrobial Activity: Zone of Inhibition (In Mm) Measured for the Wine Samples Against *E.coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*

Wine samples	Zone of Inhibition (mm)		
	<i>E.coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Streptococcus pyogenes.</i>
Grapes	23	19	15
Strawberries	21	17	12
Rice (with sugar)	18	16	15
Rice (without sugar)	13	15	11
Barley (with sugar)	14	10	10
Barley (without sugar)	09	7	12

Table.5 The Total Phenolic Compound Present in Different Wine Samples

Wine samples	TOTAL PHENOLICS (mg/ml)
Grapes	1.09
Strawberries	1.41
Rice (with sugar)	1.68
Rice (without sugar)	0.92
Barley (with sugar)	2.10
Barley (without sugar)	1.96

Sukanda Vichitphan, Kanit Vichitphan and Prapussara Sirikhansaeng, 2007 found that Krachai-Dum wines showed increase in the total phenolic content, flavonoids and total antioxidants as ageing increases but the study also suggests that the antioxidant activity depends on number of factors including the phenolic compounds present in the wine⁹. The research conducted by Korsak Towantakavanit, Yong Seo Par, Shela Gorinstein, 2010, proves that the antioxidant activity of the wine depends on the quality and ripening stages of the fruit chosen and usually increases with increase in fermentation¹⁰.

Antimicrobial Assay (Disc Diffusion Method)

Assay was performed against three bacterial strains of *E.coli*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* and the zone of inhibition produced by all the wine samples were measured (Table 4). The recent study conducted by Aleksandra N Radovanović et al supports the antimicrobial activity of the serbian red wine against various strains of bacteria by disc diffusion method¹¹.

Estimation of Total Phenolic Compounds

The total phenolic compounds was

estimated by Folin Ciocalteu method. It was observed that similar results like antioxidant concentration, strawberry wine shows the highest even for phenolic content as 1.14mg/mL. Wine from dried apricot, raisin using *S. cerevisiae* were prepared with an fermentation period of 28 days, were found to be rich in Vitamin A, lycopene, carotenes, polyphenols and antioxidants compounds¹³.

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How to cite this article:

Sasmita Sabat, Chaitra L.N. and Ranjitha, R. 2016. Evaluation of Antioxidant and Antimicrobial Activity of Wine from Various Sources. *Int.J.Curr.Microbiol.App.Sci*. 5(3): 26-35. doi: <http://dx.doi.org/10.20546/ijcmas.2016.503.004>