

Optimum Parameters for Wine Production from Fig Fruit (*Ficus racemose*) Juice

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Abstract

Wine is an alcoholic beverage made with the fermented juice of grapes. Technically, wine can be made with any fruit. Its moderate consumption may help people live longer, protect against certain cancers, improve mental health, and enhance heart health. In the present study fermentation and characterization of wine from dried *Ficus carica*(L) was studied. The ripened fruit was collected and juice was extracted was adjusted to 24°Brix, pH 3.4 and 0.8 % acidity. The juice was supplemented with 0.05 % Diammonium hydrogen phosphate (DAHP) and pasteurized at 85°C for 30min. After pasteurization, juice was inoculated with 48 hours old culture of *Saccharomyces cerevisiae* and fermentation was carried out at 24°C temperature for 20 days. The wines obtained from various treatments were evaluated for physico-chemical analysis. These wines contained 8.5°Brix TSS, 0.4 to 4.2 % total sugar, 0.2 to 4.0 % reducing sugar, 9.9 to 11.8 % alcohol. The decrease in total soluble solids, titratable acidity, reducing sugar, total sugar and increase in alcohol percentage of wine in 20 days of fermentation period was observed. Considering chemical composition, fermentation rate of wine, it may be concluded that wine prepared at 10% inoculum, 24°C temperature and 20 days of fermentation was of good quality.

Key Words: *Ficus racemosa* fruit, fermentation Juice and wine.

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I. Introduction

Ficus racemosa Linn. is a popular medicinal plant in India, which has long been used in Ayurveda, the ancient system of Indian medicine, for various diseases/disorders including diabetes, liver disorders, diarrhea, inflammatory conditions, hemorrhoids, respiratory, and urinary diseases. Fig belongs to *Moraceae* family and botanically known as *Ficus racemosa*. The fruits of *Ficus racemosa* are used for dry cough, loss of voice, disease of kidney and spleen, astringent to bowel, treatment of leucorrhoea, burning sensation, fatigue, urinary tract infection. The fruit extract has antiulcer properties, the compound β -sitosterol found in fruit possessing potent hypoglycemic activity, it reduces blood glucose level (Condit, 1951; Cheema, 1954). According to Ayurveda, fruits of *Ficus racemosa* are useful in bleeding disorders such as nasal bleeding, menorrhagia, rectal bleeding etc. Winemaking is the process of wine production, from the selection of fruits to the bottling of finished wine. Wine is an alcoholic drink typically made from fermented fruits. Yeast consumes the sugar in the juice and converts it to ethanol, carbon dioxide in approximately equal proportions and heat is liberated (Gawade, and Wasker 2003).

Wine drinking has been a part of our lives for many centuries. Wine brings pleasure to the person who would drink it. It is incontestable that wine has an innate taste that can lift one's spirit. Now a days, wine is not consumed only for pleasure. Many people make wine drinking a part of their routine because of its health benefits (Jarczyk, and Wzorek, 1977; Amerine et al., 1980). Wine is packed with anti-oxidants that help fight different types of diseases and delay the signs of aging. Wine is an alcoholic drink made from fermented fruit juice. Generally, fruits contain quantities of sugar that can be used by yeast during the fermentation process. In addition to the inherent characteristics of fruit (pH values, sugar contents and nitrogen contents), other factors must be taken into account during fruit wine production (Amerine and Kunkee 1968).

Fruit wines are undistilled alcoholic beverages usually made from grapes or other fruits which are nutritive, more tasty and mild stimulants. These fruits undergo a period of fermentation and ageing. They usually have an alcohol content ranging between 5 to 13%. Fruit wine contains most of the nutrients present in the original fruit juice along with contain alcohol, vitamin and other compounds synthesized by the fermenting yeast and some compounds added during the manufacture of wine (Bhutani et al., 1989).

An efficient wine production requires fermentable carbohydrates, an efficient yeast strain, a few

nutrients and simple culture conditions. At the moment, most of the wine production processes are relying on *Saccharomyces cerevisiae* strains that allow rapid and reliable fermentations, reduce the risk of sluggish or stuck fermentations and prevent microbial contaminations (Kamble, 1998; Romano *et al.*, 2003). Yeast starter cultures that are specifically selected for the winemaking process on the basis of scientifically verified characteristics typically complement and optimize the raw material quality and individual characteristics of the wine, producing a more desirable product (Swiegerset *et al.*, 2005; Chakraborty *et al.*, 2017). In the present study the production and the analysis of inoculum size, TSS, temperature, pH, substrate concentration and incubation period of Wine from Fig Fruit (*Ficus Carica*) were studied.



Fig.1. *Ficus racemosa* plant. (Cluster fig)



Fig. 2. *F. racemosa* fruits

II. Material And Method

Collection of Fruits: Fully-ripened, healthy fruits were collected and used for preparation.

Yeast culture: The yeast strain *Saccharomyces cerevisiae* (MCC-...) was obtained from National Centre for Cell Science, Pune and taken as standard strain. The cultures were kept in refrigerator until used.

Preparation of inoculums: The yeast inoculum was sub-cultured once in a month. Malt- Glucose-Yeast-Peptone medium (MGYP) containing 0.3 % malt extract, 1.0 % glucose, 0.3 % yeast extract, 0.5 % peptone and 2.0 % agar was prepared. After adjusting the pH between 6.4 to 6.8, the medium was autoclaved at 15 lbs pressure for 20 minutes and made into slants. After cooling, the slants were inoculated with a loopful of pure yeast culture and incubated at 37°C for 24-48 hours. They were stored in the refrigerator at 5°C until used.

Preparation of juice: The fresh fruits of *Ficus racemosa* (Fig) were collected from the fruit vendor shop Nashik, Maharashtra. The juicy arils were separated from fresh fruits with the help of stainless-steel knife. The arils were crushed to juice in fruit mixer. 700 ml of juice was collected from 2 kg of *Ficus racemosa* (Fig) fruit. The juices were transferred into five 250ml conical flask with each conical flask containing 100ml of juice. The inoculum was added to juice and allowed for fermentation.

Extraction of juice:

The fresh fruits of *Ficus racemosa* (Fig) were collected from the fruit vendor shop Nashik, Maharashtra. The fruits were washed and cleaned thoroughly with running water and subjected to blanching at 82°C for 5 minutes. The fruit was crushed and water was added 1.5 times of the fruit. Pulp was pressed manually through muslin cloth to obtain clear juice. Pasteurization of juice was done at 85°C for 30 min followed by immediately cooling under tap water. Then the clear juice was used for chemical analysis and for preparation of wine.

Analysis of juice

The juice was analyzed for Total Soluble Solids, pH, total sugars, reducing sugar, non-reducing sugar and protein content.

Total soluble solids (TSS)

The content of total soluble solids (TSS) in the juice was determined with the help of Erma hand refractometer. Care was taken that the prism of refractometer was washed with distilled water and wiped dry before every reading.

The total soluble solid content of juice was adjusted to 24°Brix by addition of sucrose (cane sugar).

pH: pH of the juice was determined by pH meter and adjusted to 3.4 by the addition of tartaric acid.

Reducing sugar by DNSA method

Prepare 20 mL of 2N NaOH. Weigh 1 g DNSA and dissolve in 20 mL NaOH with the help of a magnetic stirrer. Weigh 30 g of sodium potassium tartrate and dissolve in 50 mL dH₂O. Slowly pour sodium potassium tartrate solution in the DNSA and NaOH solution and made the volume up to 100 ml. Take 7 tubes and label them as Blank, Test and 1 to 5. Make dilutions of glucose standards upto 1ml in 1-5 tubes with distilled water, 1ml of unknown sample in test and 1ml of distilled water in blank. Add 1 ml of DNSA reagent to all the seven test tubes. Mix well. Keep in boiling water bath for 15 minutes. Then add 8ml of distilled water and record the absorbance with a spectrophotometer at 540nm. First, take the absorbance (OD) of Blank and make it zero. Take the OD of all the tubes. Wash the cuvettes each time after taking OD. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of sugar present in the sample tube.

Total sugar by Anthrone's method

Dissolve 200mg of Anthrone in 100ml liter of concentrated H₂SO₄. Use freshly prepared reagent for the assay. Take 7 tubes, prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8, 1mL of the working standard and 1ml of unknown sample. '0' serves as blank. Make up the volume to 1mL in all the tubes by adding distilled water. Then add 4mL of anthrone reagent. Heat for eight minutes in a boiling water bath. Cool rapidly and read the green to dark green color at 630nm. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrate present in the sample tube.

Non reducing sugar

Non reducing sugar can be determined by the difference between total sugar and reducing sugar.

Protein estimation by Folin-Lowry method

Take 7 tubes, prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8, 1mL of the working standard and 1ml of unknown sample. '0' serves as blank. Make up the volume to 1mL in all the tubes by adding distilled water. Then add 5mL of alkaline reagent in all tubes. Incubate tubes for 10 minutes. Add 0.5ml FC reagent in all tubes and incubate in dark for 30 minutes. Observe for blue color and record the absorbance with a spectrophotometer at 660nm. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of protein present in the sample.

Preparation of inoculum

The juice was supplemented with 0.05 % diammonium hydrogen phosphate (DAHP) and potassium metabisulphite (KMS) equivalent to 50 ppm SO₂. After adjusting pH, TSS, DAHP and KMS of the juice, 50ml of juice was taken in conical flask and pasteurized at 82-85°C for 30 min. Pasteurized juice, after cooling, was incubated with *Saccharomyces cerevisiae*. The flask was incubated at 24°C for 48 hours. This inoculum was used for the wine production.

Fermentation of fruits juice of *Ficus racemosa*

1. Inoculum level: 10%
2. Fermentation period: 20 days
3. Temperature of fermentation: 24°C
4. Yeast strain used for preparation of inoculum: *Saccharomyces cerevisiae*

Analysis of wine

The wine obtained after fermentation of 20 days was analyzed for total soluble solid, amount of reducing sugar, total sugar and amount of protein were estimated by the standard procedures as described under analysis of juice. Alcohol content of wine was analyzed by potassium dichromate method.

Estimation of alcohol

Take 1ml different concentrations of standard ethyl alcohol (2-16%), 1ml of wine sample and 1ml of distilled water for blank in 50 ml of 10 volumetric flask. In all the flasks, the volume was made to 5 ml with distilled water. Then 25ml of dichromate reagent was added in each flask and the flasks were heated at 60°C for 20 minutes in a hot water bath. After cooling, the volume was made to 50ml with distilled water. The intensity of color was measured at 600 nm. A graph of ethyl alcohol concentration vs. absorbance was drawn.

Wine adjustment:

pH: The pH of wine was adjusted to 3.4 by adding tartaric acid.

SO₂ level: The SO₂ of wine was adjusted to 20 ppm.

Clarification of wine:

Fining of wine: Fining is commonly used to accelerate the spontaneous precipitation of suspended material. Fining agents bind to or adsorb particulate matter. The aggregates are generally sufficiently large to precipitate quickly. 0.1% bentonite was added to wine and the wine was allowed to settle for overnight.

Centrifugation: The wine was centrifuge after fining with bentonite. Centrifugation of wine employs rotation at high speed for settling suspended particles. It is equivalent to spontaneous sedimentation, but occurs within minutes rather than months.

Filtration: The wine was then filtered with vacuum filter to obtain clear wine and the wine was then stored in bottle.

III. Result And Discussion

By the analysis of juice and wine the results obtained are briefly presented in this section.

Parameter	Content
A. Fig fruit:	
Color	Reddish green
Average weight (gm)	21.43
B. Chemical composition of juice:	
TSS (°Brix)	8
pH	4.4
Sugars:	
-Reducing sugars (mg/ml)	8.81
-Non-reducing sugars (mg/ml)	3.8
-Total sugar (mg/ml)	12.61

Analysis of fruit juice and wine

The wine and fruit juice samples were analyzed for TSS, total sugar, reducing sugar, protein and alcohol content.

Sample	TSS		Reducing sugar (mg/ml)	Total sugar(mg/ml)	Protein (µg/ml)
	before fermentation	after fermentation			
Fruit juice	before fermentation	after fermentation	8.81	12.61	394.12
Wine	24°Brix	11°Brix	4.05	5.81	322.25

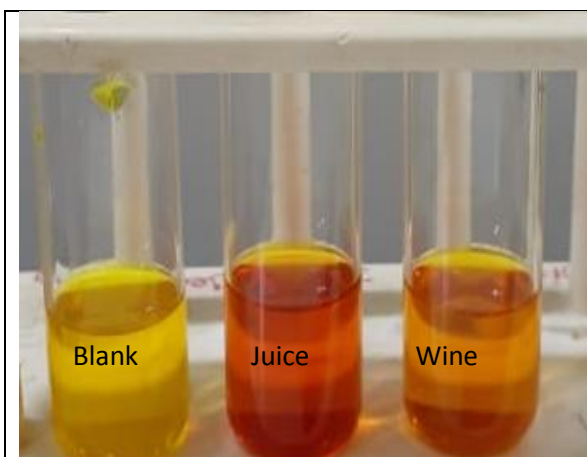


Fig.3. Estimation of reducing sugar by DNSA Method

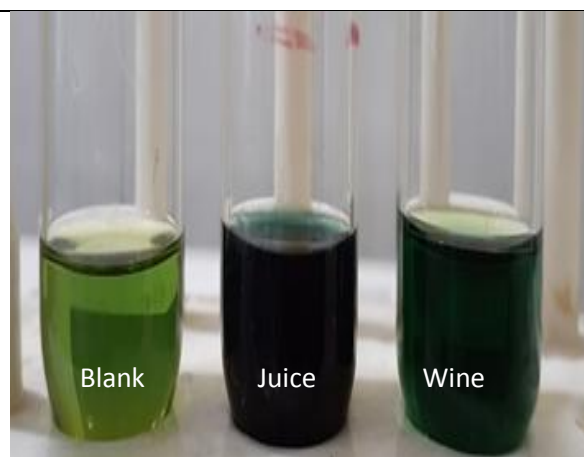


Fig.4. Estimation of total sugar by Anthrone's Method

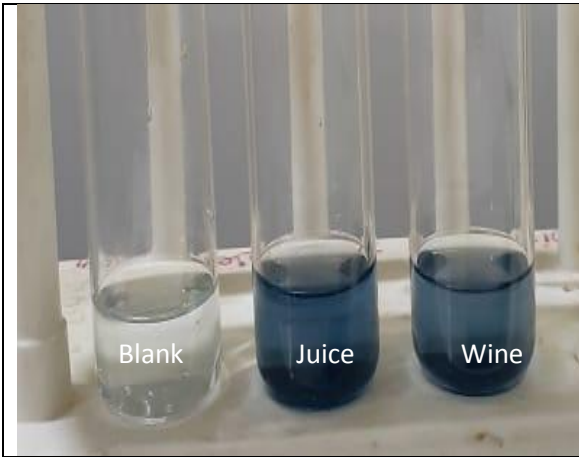


Fig.5. Estimation of protein by Folin-lowry method.



Fig.6. Estimation of alcohol by Dichromate method

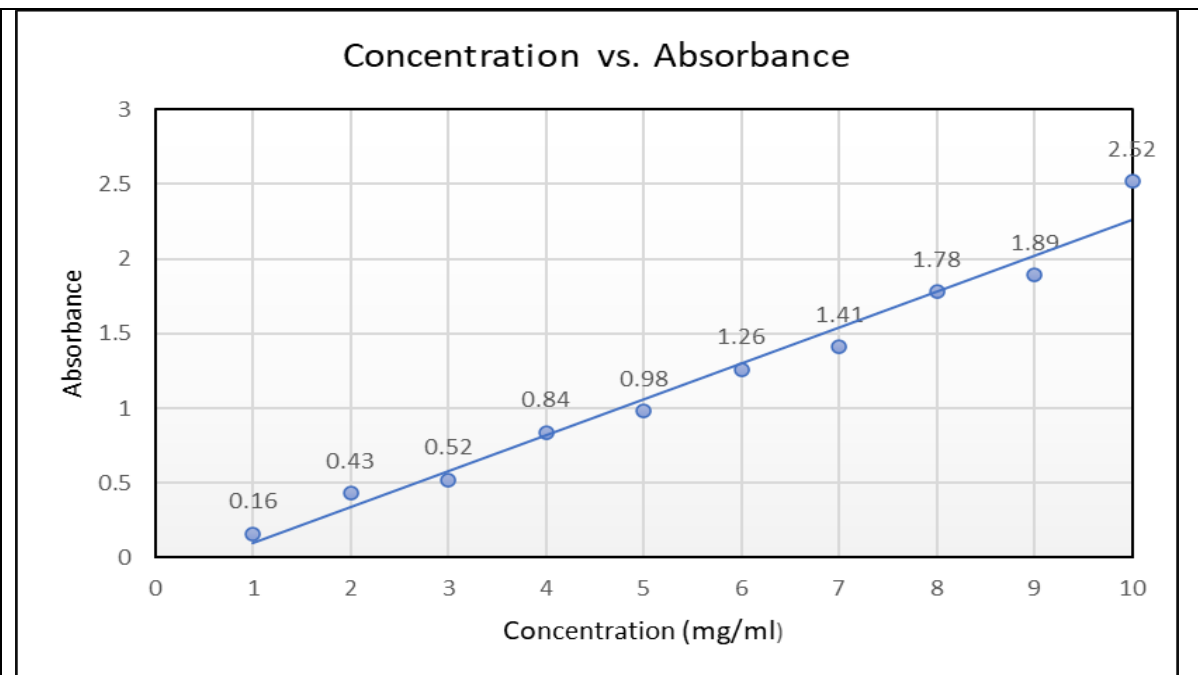


Fig.7. Standard graph of concentration of reducing sugar vs. absorbance

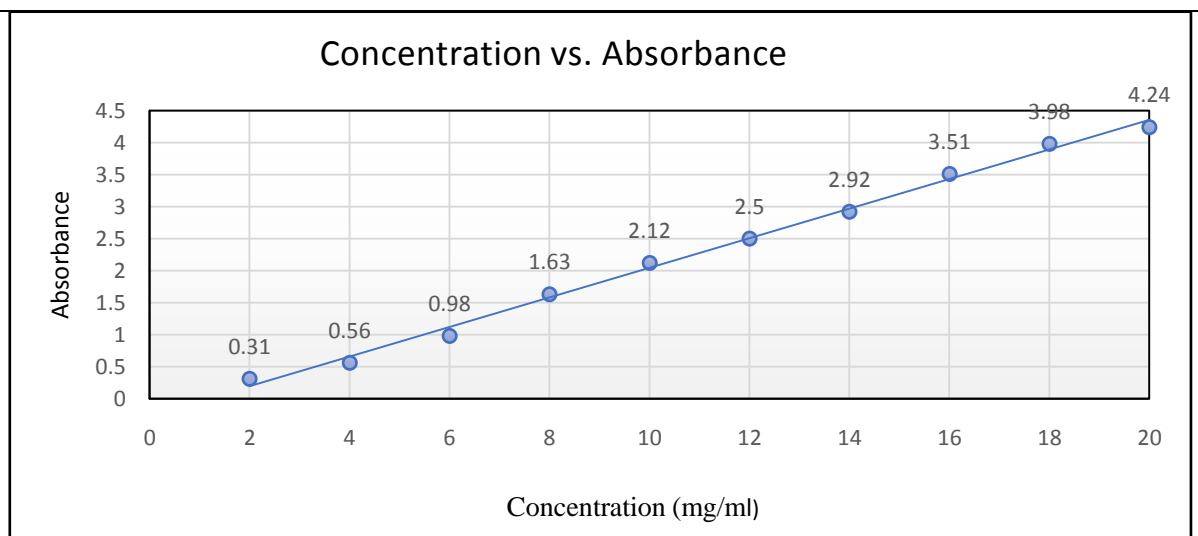


Fig.8. Standard graph of concentration of total sugar vs. absorbance

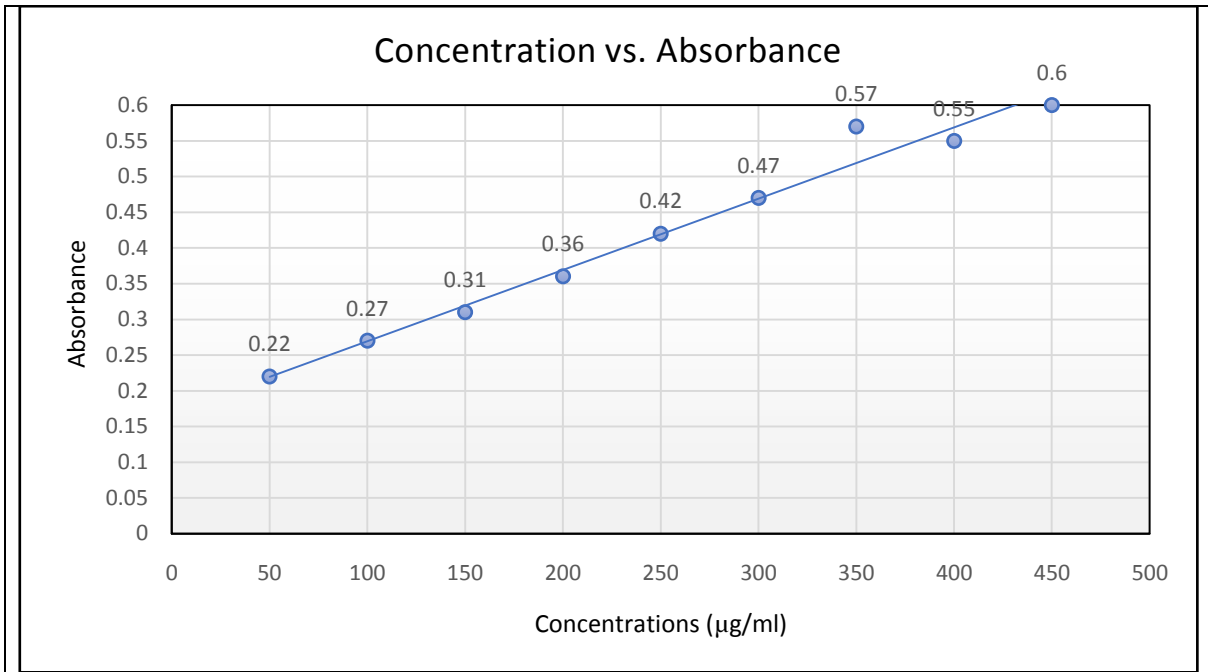


Fig.9. Standard graph of concentration of protein vs. absorbance

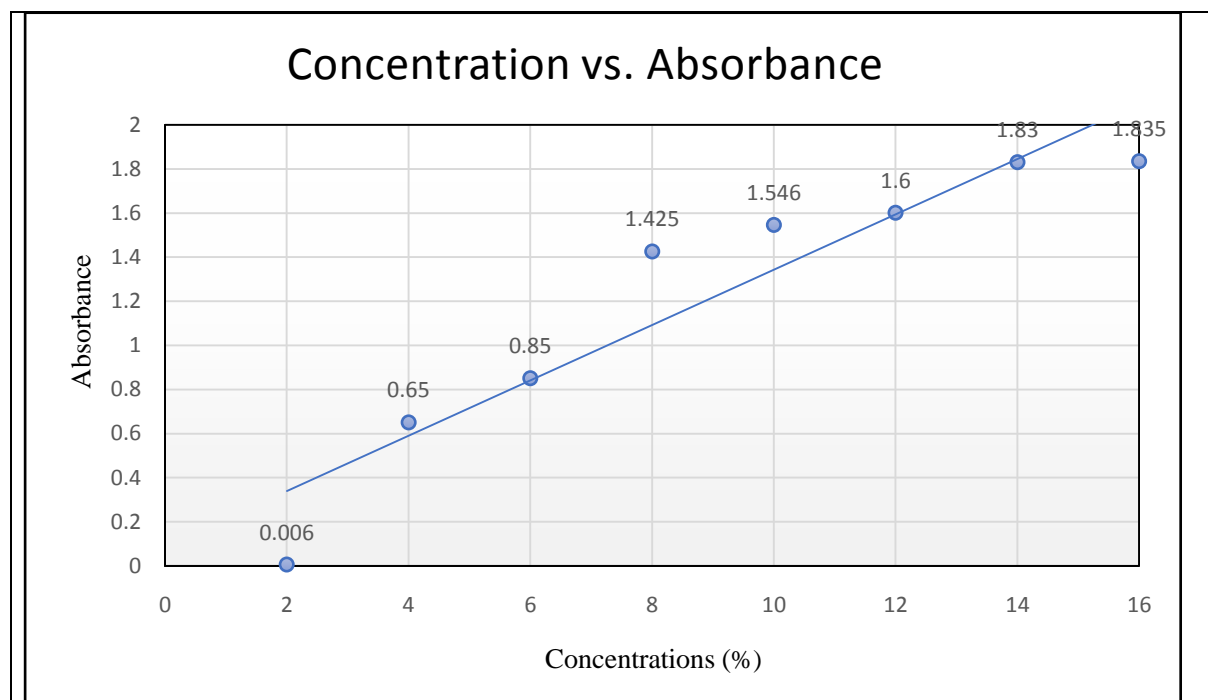


Fig.10. Standard graph of Concentration of alcohol vs. absorbance

The amount of total carbohydrate in the juice and in wine were 12.61mg/ml and 5.81mg/ml, respectively. The concentrations of protein in the juice and wine were 394.12 µg/ml and 322.25 µg/ml, respectively. The minimum total sugar content (0.4%) was observed in 10% inoculums 20 days wine sample. Total sugar is degraded to sucrose and maltose which in turn converted to glucose and fructose and made available to yeast (Manner, 1971). Interaction effect between inoculum level and fermentation period was found to be insignificant.

However, effect of inoculum level is found to be significant. Also effect of fermentation period was found to be significant. The concentration of alcohol in the wine was 11.14%. The TSS content of wine depends upon the degree of fermentation. The TSS content of wines prepared from different fruit juice has been reported to be 5.0 to 14.8 % (Pathan *et.al.* 2003). According to Jawahar (1999), the wines were prepared by adjusting TSS level of 16, 18, 20, 22 and 24°Brix the wine contained 5.0 to 8.0°Brix. The dry wine contained 0 to 1%

sugars, while semi-dry, sweet and very sweet wines contained 2 to 3, 8 to 11 and more than 12 % sugars, respectively (Jarczyk and Wzorek, 1977). Nikrad (1993) reported 6.77 to 11.23 % total sugar, 6.13 to 10.03 % reducing sugar and 0.17 to 1.23 % non-reducing sugar in pomegranate wine, while Bardiyat *et al.*, (1974) reported 7.8 and 10 % reducing sugars in guava juice wine and guava pulp wine, respectively. Sulfur dioxide is generally used in winemaking (generally 50-100 mg/L) and has two functions. The first is to inhibit or kill the natural microflora (bacteria and yeasts) in the juice and thus facilitate the activity of the selected yeasts added to the must. The wines obtained from various treatments were evaluated for physico-chemical analysis. These wines contained 8.5°Brix TSS, 0.4 to 4.2 % total sugar, 0.2 to 4.0 % reducing sugar, 9.9 to 11.8 % alcohol. The decrease in total soluble solids, titratable acidity, reducing sugar, total sugar and increase in alcohol percentage of wine in 20 days of fermentation period was observed.

IV. Conclusion

The fermented wine from fig is prepared at 10 % inoculum and 20 days of fermentation. However, no significant change in sensory properties was seen due to different inoculum percentages and fermentation period. Considering chemical composition, fermentation rate and sensory properties of wine prepared by various treatments it may be concluded that wine prepared at 10% inoculum, 24°C temperature and 20 days of fermentation was of good quality.

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