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## Medicinal Importance of Pomegranate wine



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### ABSTRACT

A Pomegranate wine contains powerful antioxidants that inhibit the onset of atherosclerosis, reduce the risk of heart disease, and mediate high blood pressure. By fermentation process, Phenolic compounds present in seed & skin of fruit can be drawn which are absent in juice. As the Phenol concentration of the wine increases, more efficiently it will work as antioxidant sources which will be a great add to the medicine world and as well as healthy beverage industry. Wine can be preserved for years without losing its antioxidant activity. It overcomes the problem of seasonal availability. Pomegranate wine was prepared at laboratory scale and evaluated. This wine is compared with fruit juice for its Sugar content 6% (in fruit juice-15%), Carbohydrate content 176 ug/100 ml ( in fruit juice-246 ug/100 ml), Protein content 88 ug/100 ml(in fruit juice 119 ug/100 ml), alcohol content 8.89% (absent in fruit juice). Because of the reduced sugar level in wine, it is suitable for the diabetic patient. For diabetic patients, the dry wine is very effective because it has more alcohol content and due to this it reduces the concentration of Sugar in the wine and more extraction of phenolics which are very much effective.



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## INTRODUCTION

India is rich in the cultivation of pomegranate fruit *Punica granatum*. Red fruits like pomegranate contain polyphenolic compounds having good antioxidant activity inhibit the onset of atherosclerosis, reduce the risk of heart disease, and mediate high blood pressure. Pomegranate extract also has demonstrated anticarcinogenic properties that are effective in suppressing a variety of cancers, including skin, breast, and colon cancers. The pomegranate has even shown effectiveness in alleviating depression in a mouse model of menopause. Epidemiological studies show that consumption of fruits and vegetables with high phenolic content correlate with reduced cardiovascular and cerebrovascular diseases and cancer mortality. It reduces the hardening of arteries and hence the risk of heart attack & also reduces the risk of atherosclerosis.

The pomegranate tree is native from Iran to the Himalayas in northern India and has been cultivated since ancient times throughout the Mediterranean region of Asia, Africa, and Europe. The fruit was used in many ways as it is today and was featured in Egyptian mythology and art, praised in the Old Testament of the Bible and in the Babylonian Talmud, and it was carried by desert caravans for the sake of its thirst-quenching juice. It traveled to central and southern India from Iran about the first century A.D. and was reported growing in Indonesia in 1416. It has been widely cultivated throughout India and drier parts of south-east Asia, Malaya, the East Indies and tropical Africa. The most important growing regions are Egypt, China, Afghanistan, Pakistan, Bangladesh, Iran, Iraq, India, Burma and Saudi Arabia.

### Morphology

Pomegranates, '*Punica granatum*,' are dense, bushy shrubs 6 to 12 feet tall with thorny, slender branches that may be trained into small trees. Orange-red flowers appear on new growth in the spring and summer and are bell-shaped and vase-shaped. The vase-shaped flowers are normally sterile, so they will not develop into fruit. The fruit contains numerous seeds surrounded by sweet, pink, juicy, tasty pulp covered with leathery-brown to red, bitter skin, which is easily peeled. This juice was used by the ancients as an ink or dye, because of its persistence in staining permanently.

## MATERIALS & METHODS

### Fermentation

The fruit was peeled manually to separate out the crimson colored seeds. Rotten part of the fruit was removed and the best part was selected. The selected part had been crushed. Potassium metabisulphite, water, and sugar were added. The normal dose is one crushed and dissolved Campden tablet to each gallon of must, or 1/4 teaspoon of potassium metabisulfite to each 5 gallons of must. Do not add more than this, as too much is in some cases worse than not enough. Potassium metabisulphite Used to increase the aging life of wine by adding only 1/4 teaspoon to the full 6 gallons during the final racking.

When used in place of Campden Tablets for sulfite additions to wine, 1/4 tsp per 5 gallons which yields 40-45ppm. The solution was stirred thoroughly.

The must was placed aside for 10 hours so that all the live organisms could be eradicated.

The next day Pectic enzymes are added, stirred well and refrigerate for 24hrs to maintain the temp of Must between 10 – 16 degree celsius.

Yeast nutrients contain a variety of trace minerals, but especially nitrogen. For a sterile must 1-1/2 to 2 teaspoons of nutrients per gallon of must might be required, but for most wines made from other than wine grapes, a teaspoon of nutrients is sufficient. Yeast should not be baking Recipes most generally call for the addition of acid blend, which is a mixture of citric, malic and tartaric acids in crystalline form. Yeast was added as starter solution at room temperature. Cultures are available in several forms, but the most convenient form for the average home winemaker is in the Active Dry Yeast (ADY) form. The yeast is in a dehydrated, dormant state yet very much alive. The most convenient quantity is the 5-gram foil packet, or sachet, suitable for quickly inoculating one to five gallons of must or for making an activated starter. These same packets can be used to inoculate larger batches, but it may take several extra days for the culture to multiply sufficiently. In theory, one yeast cell is all that's required to inoculate a 10,000-gallon vat, but it takes too long to breed the required density and the must could spoil long before such a density is achieved.

Fermentation was allowed at 20-25 degree celsius without an airlock because it needs Oxygen exposure for 48-72 hours. After 3 days the must was transferred to a secondary vessel by siphoning. The secondary vessel was fitted with airlock for this a pipe coming out of the vessel was dipped into a container containing water so that no outer air could enter the vessel and CO<sub>2</sub> produced in the vessel could come out of it by replacing the water in the vessel. Fermentation is carried out in an anaerobic condition and temperature was maintained at 15°C-19°C till sugar percentage comes down to 6 Brix.

Today the accepted practice is to keep the wine in the primary until the vigorous fermentation subsides. This normally occurs at around specific gravity 1.010. The best way to accomplish the transfer without exposing the wine to more oxygen than is necessary is to tilt the funnel so that the wine escaping it slides down the inside of the carboy rather than drop free-fall through the air to the bottom. This may require the tilting of the carboy itself, but simply tilting the funnel means this is a two-person operation. An alternative procedure is to fit a hose or tubing to the bottom of the funnel that extends to the bottom of the carboy. This, too, minimizes air contact. It sometimes happens that everything in the must is just perfect for the yeast and they ferment the must to absolute dryness in only two or three days. When this happens (a hydrometer reading will show the specific gravity at less than 1.000), go ahead and rack the wine into the secondary and slap an airlock on it immediately. When the fermentation in the secondary stops that is, when the positive pressure inside the carboy stops pushing bubbles through the airlock, it is essential to use the hydrometer to ensure fermentation is finished rather than stuck.

**Table 1:** During secondary fermentation change in pH, bricks, and specific gravity was recorded.

Days	pH	Bricks	Specific gravity
Day 1	2.69	21	1.012
Day 2	2.60	20	1.011
Day 3	2.60	18	1.011
Day 4	2.58	16	1.011
Day 5	2.53	15	1.009
Day 6	2.61	13	1.009
Day 7	2.52	13	1.009
Day 8	2.53	12	1.005
Day 9	2.48	10	1.004
Day 10	2.51	8	1.004
Day 11	2.43	8	1.003
Day 12	2.42	7	1.000
Day 13	2.43	7	1.000
Day 14	2.44	6	1.000
Day 15	2.42	6	1.000

Evaluation of Wine: Chemical analysis,

### 1. Estimation of Total Carbohydrate

#### Phenol Sulphuric Acid Method for Total Carbohydrate

##### Principle

In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a green coloured product with phenol and has an absorption maximum at 490nm.

##### Materials

1. Phenol 5%: Redistilled (reagent grade) phenol (50gm) dissolved in water and diluted to one litre.
2. Sulphuric acid 96% reagent grade.

3. Standard Glucose: Stock – 10mg in 100ml of water. Working standard -10ml of stock diluted to 100ml with distilled water.

**Procedure:**

1. Weigh 10mg of the sample into boiling tube.
2. Hydrolyse by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCL and cool to room temperature.
3. Neutralise it with solid sodium carbonate with the effervescence ceases.
4. Make up the volume to 100ml and centrifuge.
5. Pipette out 0.2, 0.4, 0.6 0.8 and 1ml of the working standard in to a series of test tubes.
6. Pipette out 0.1 and .2ml of the sample solution in two separate test tubes. Make up the volume in each tube to 1ml with water.
7. Set a blank with 1ml of water.
8. Add 1ml of phenol solution to each tube.
9. Add 5ml of 96% sulphuric acid to each tube and shake well.
10. After 10min shake the contents in the tube and place in a water bath at 25-30°C for 20min.
11. Read the colour at 490nm.
12. Calculate the amount of total carbohydrate present in the sample solution using the standard graph.

**2. Protein Estimation by Lowry's Method**

**Principle:**

The blue colour developed by the reduction of the phosphomolybdic-phosphotungstic components in the Folin-Ciocalteu reagent by the amino acids tyrosine and the tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured by the Lowry's method.

**Materials:**

1. 2% Sodium Carbonate in 0.1N Sodium Hydroxide (Reagent A).
2. 0.5% Copper Sulphate (CUSO<sub>4</sub>.5H<sub>2</sub>O) in 1% potassium sodium tartrate (Reagent B)

3. Folin-Ciocalteu Reagent (reagent D)- Reflux gently for 10 hrs a mixture consisting of 100g sodium tungstate ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ), 25g sodium molybdate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ), 700ml water, 50ml of 85% phosphoric acid, and 10ml of concentrated hydrochloric acid in a 1.5l flask. Add 150g lithium sulfate, 50ml water and a few drops of bromine water. Boil the mixture for 15min without condenser to remove excess bromine. Cool, dilute to 1l and filter. The reagent should have no greenish tint. (Determine the acid concentration of the reagent by titration with 1N NaOH to Phenolphthalein end-point).

#### 4. Protein Solution (Stock Standard)

Weigh accurately 50mg of bovine serum albumin (Fraction 5) and dissolve in the distilled water and makeup to 50ml in a standard flask.

#### 5. Working Standard

Dilute 10ml of the stock solution to 50ml with distilled water in a standard flask. 1ml of this solution contains 200ug protein.

### Procedure:

#### Estimation of protein

1. Pipette out 0.2, 0.4, 0.6, 0.8, and 1ml of the working standard into a series of test tubes.
2. Pipette out 0.1ml and 0.2ml of the sample extract in two other test tubes.
3. Make up the volume to 1ml in all the test tubes. A tube with 1ml of water serves as the blank.
4. Add 5ml of reagent C to each tube including the blank. Mix well and allow to stand for 10min.
5. Then add 0.5ml of reagent D, mix well and incubate at room temperature in the dark for 30min. the blue colour is developed.
6. Take the reading at 660nm.
7. Draw a standard graph and calculate the amount of protein in the sample.

### 3. Phenols

Phenols, the aromatic compound with hydroxyl groups are widespread in the plant kingdom. They occur in all parts of the plants. Phenols are said to offer resistance to disease and pests in plants. Grains containing a high amount of polyphenols are resistant to bird attack. Phenols

include an array of compounds like tannins, flavonols etc. Total phenols estimation can be carried out with the Folin-Ciocalteu reagent.

**Principle:**

Phenols react with the phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium and produce blue coloured complex (molybdenum blue).

**Materials:**

1. 80% ethanol
2. Folin-Ciocalteu reagent
3. Na<sub>2</sub>CO<sub>3</sub>, 20%
4. standard (100mg Catechol reagent in 100ml water)

dilute 10 times for working standard.

**Procedure:**

1. Weigh exactly 0.5 to 1.0g of the sample and grind it with a pestle and mortar in the 10-time volume of 80% ethanol.
2. Centrifuge the homogenate at 10000rpm for 20min. Save the supernatant. Re-extract the residue with five times the volume of 80% ethanol, centrifuge and pool the supernatants.
3. Evaporate the supernatant to dryness.
4. Dissolve the residue in a known volume of distilled water (5ml).
5. Pipette out different aliquots (0.2 to 2ml) into test tubes.
6. Make up the volume in each tube to 3ml with water.
7. Add 0.5ml of Folin-Ciocalteu reagent.
8. After 3min, add 2ml of 20% Na<sub>2</sub>CO<sub>3</sub> solution to each tube.
9. Mix thoroughly. Place the tubes in a boiling water exactly 1 min, cool and measure the absorbance at 650nm against a reagent blank.
10. Prepare a standard curve using different concentrations of catechol.



**Calculations:**

From standard curve find out the concentration of phenols in the test sample and express as mg phenols/100g material.

**4. Estimation of amount of alcohol (Ceric ammonium nitrate method)**

**Principle**

Alcohol and phenols are capable of replacing nitrate ion from the complex ceric ammonium nitrate. This reaction results in a colour change from yellow to red and can be estimated calorimetrically.

**Requirement**

1. Distillate
2. Ceric ammonium nitrate reagent
3. Known std. alcohol solution (1gm %)
4. Calorimeter adjusted at 540nm

**RESULTS & DISCUSSION**

**1. Protein:** estimation by Folin lowry's method-

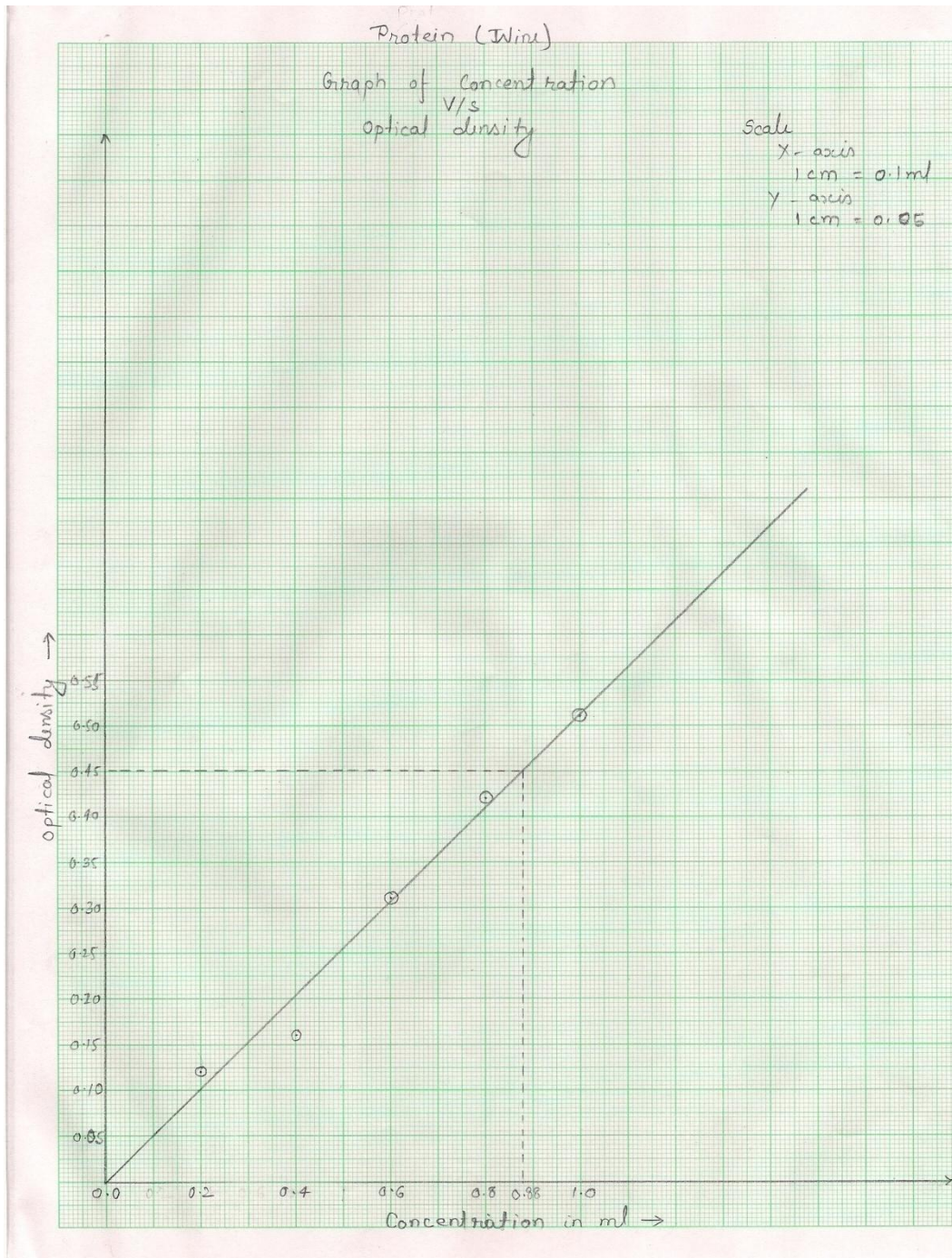
**Table No 2: Protein estimation by Folin lowry's method**

Reagent	Blank	1	2	3	4	5	UK
Std.	-	0.2	0.4	0.6	0.8	1.0	-
UK	-	-	-	-	-	-	1ml
D/W	1	0.8	0.6	0.4	0.2	-	-
Alkaline	4	4	4	4	4	4	4
Folin-	0.5	0.5	0.5	0.5	0.5	0.5	0.5

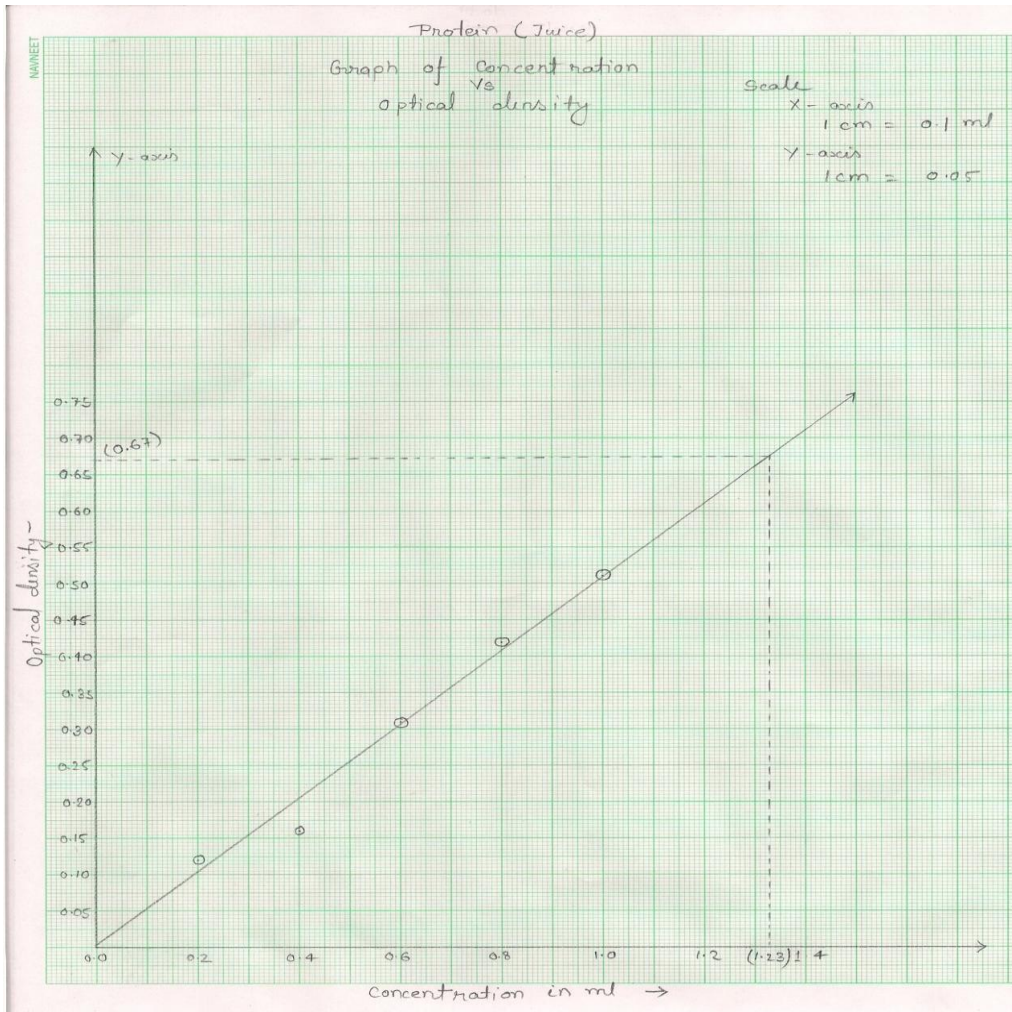
Keep the tubes at room temperature for 30 min.

**Table No 3: Optical Density measurement.**

O.D at	0.0	0.12	0.17	0.32	0.42	0.51	0.45
O.D at	0.0	0.12	0.17	0.32	0.42	0.51	0.67



**Graph 1: From the graph unknown concentration is 0.88, 1ml = 200 ug, 0.88 ml = X, X = 0.88x200 = 176ug in 100 ml of sample.**



**Graph 2:** From the graph unknown concentration is 1.23, 1ml = 200 ug, 1.23 ml = X, X = 1.23x200 = 246ug in 100 ml of sample.

**2. Carbohydrate:** Phenol Sulphuric Acid Method for Total Carbohydrate

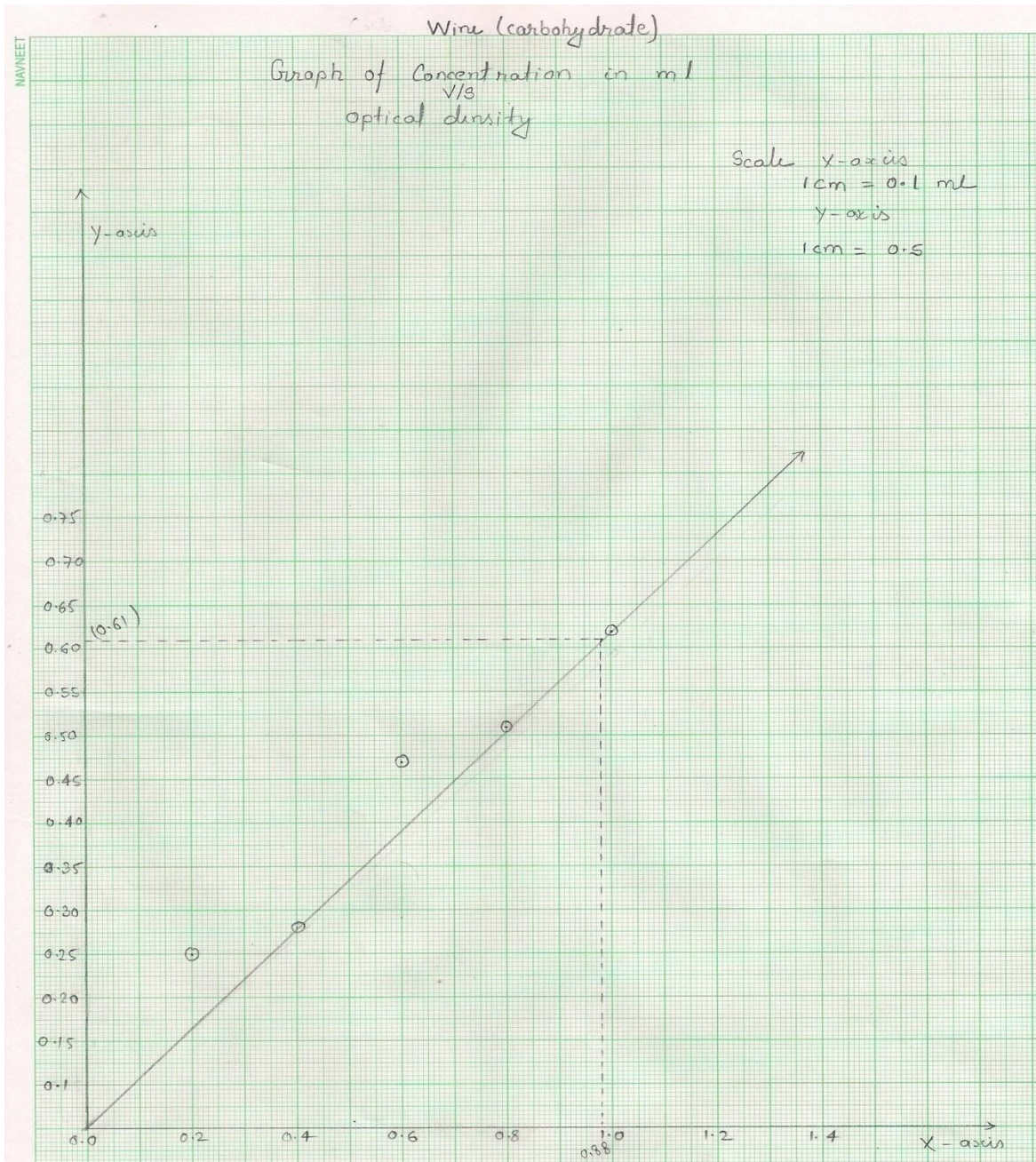
**Table No 4: Estimation of Total Carbohydrate**

Reagent	1	2	3	4	5	S1	S2	Blank
Std.	0.2	0.4	0.6	0.8	1.0	0.1	0.2	-
UK	-	-	-	-	-	-	-	-
Phenol Solution	1	1	1	1	1	1	1	-
96% H <sub>2</sub> SO <sub>4</sub>	5	5	5	5	5	5	5	-

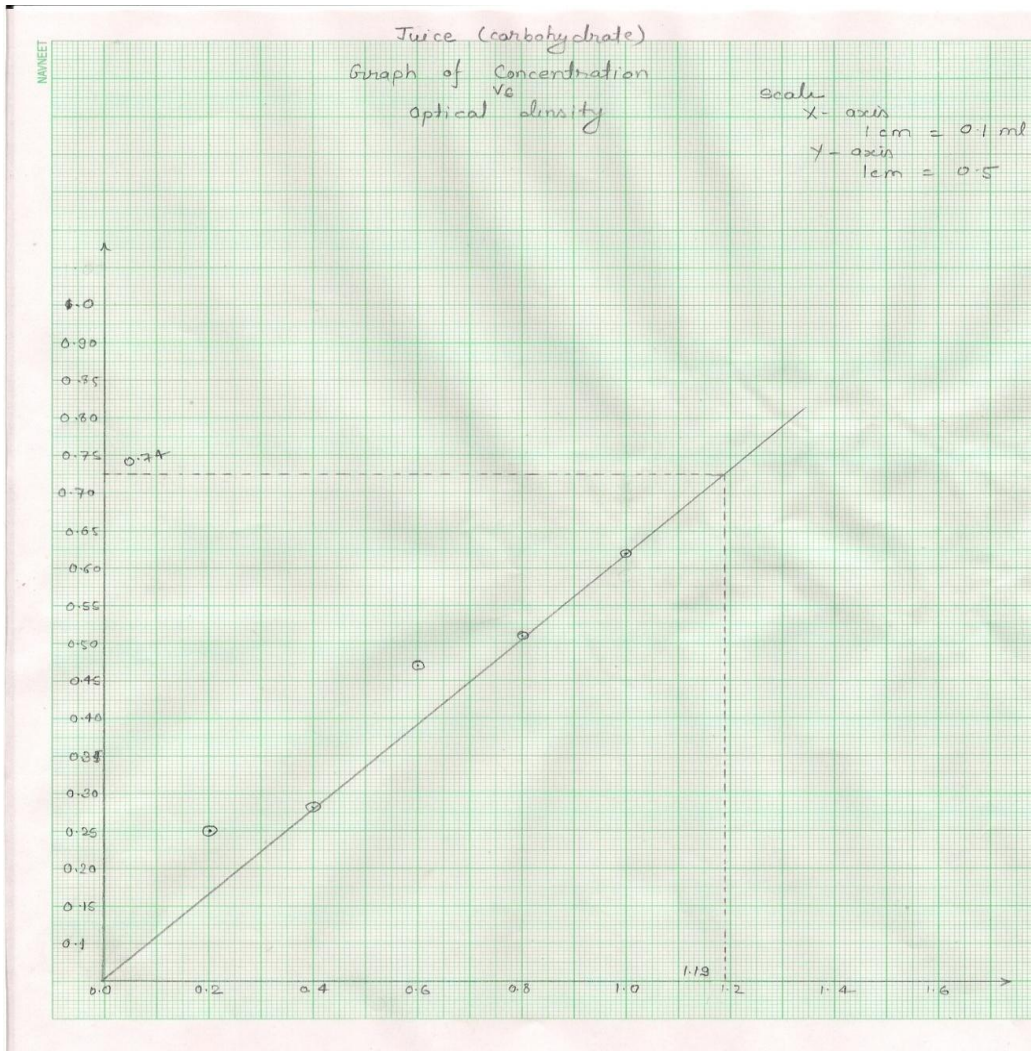
After 10 minutes shake the content and place in water bath 25-30<sup>0</sup>c for 20 min

**Table No 5: Optical Density measurement.**

OD	0.25	0.28	0.47	0.51	0.58	1.40	2.42	-
OD	0.25	0.28	0.47	0.51	0.58	1.40	1.80	-



**Graph 3: From the graph unknown concentration is 0.88, 1ml = 100 ug, 0.88 ml = X , X = 0.88x100 = 88ug in 100 ml of sample.**



**Graph 4:** From the graph unknown concentration is 1.19, 1ml = 100 ug, 1.99 ml = X , X = 1.99x100 = 199ug in 100 ml of sample.

**3. Alcohol:** Ceric ammonium nitrate method:

**Table 6: Alcohol Content**

	Test	blank	Std.
DW	-	2.5	-
Std. alcohol	-	-	2.5
Distilled water	2.5	-	-
Reagent	2.5	2.5	2.5

Mix well & incubate at RT for five minutes, Result: % yield of alcohol was found 8.89 %

**Table 7: Optical density measurement**

OD at 540nm	0.8	0.00	0.09
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**Comparison of results:**

**Table 8: Comparison of Result.**

Biomolecules	Fruit juice	Wine
Sugar	15%	6%
Carbohydrate	246 ug/100 ml	176 ug/100 ml
Protein	119 ug/100 ml	88 ug/100 ml
Alcohol (%)	-	8.89

**DISCUSSION**

With the help of fermentation we are able to draw most of the Phenolics compound present in the seed and skin of the fruit, which is not present in the juice. As the Phenol concentration of the wine increases, more efficiently it will work as antioxidant sources which will be a great add to the medicine world and as well as healthy beverage industry. Wine can be preserved for years without losing its antioxidant activity. It overcomes the problem of seasonal availability. Also because of the reduced sugar level in wine, it is also suitable for diabetics. For diabetic patients, the dry wine is very effective because it has more alcohol content and due to this it reduces the concentration of Sugar in the wine and more extraction of phenolics which are very much effective.

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