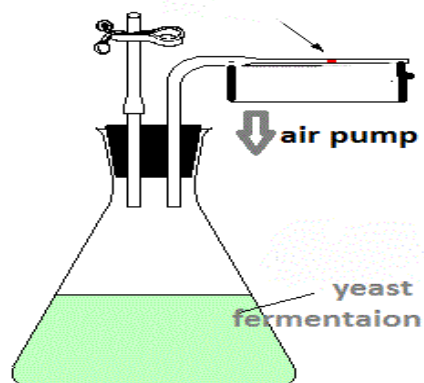
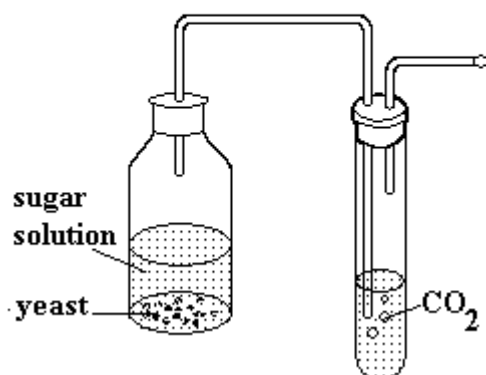


Experimental method:

TO PREPARATION OF ETHANOL (date extract medium):

1. Mix 100 gm from date with 100 ml tap water, heat at 80 C^o for 30 min.
2. By Gauze filter the mixture or the Soaked.
3. Inoculate the medium (date extract medium) with 1% *saccharomyces cerevisiae* culture grown in malt extract broth & incubate at 30 co for 48 hrs in flask incubator has air pump to convert the condition to anaerobic which is suitable for ethanol production.

Yeast reacting with sugar solution



Detection of ethanol by chemical methods:

1. Ceric ammonium nitrate (CAN) method:-

- a) Take 0.5 ml of CAN, add 3ml of D.W to it (dilution).
- b) Add 3-5 drops from the sample (yeast fermented medium) to diluted CAN → the appearance of red color indicate to a presence of ethanol .

2. potassium dichromate method :-

- a) Add 5 ml of $K_2Cr_2O_7$ solution to 1 ml of concentrated sulfuric acid & heat the mixture.
- b) Add 1 ml of the sample (yeast fermented medium) to the mixture. The positive result: is appearance of green color with acetaldehyde odor.

Preparation alcohol in laboratories and factories:

In this method, use a person square to determine the proportion of sugary substance in the preparatory solution.

We need to:

- 1-water
- 2- raw sugary substance
- 3-yeast culture or vinegar mother
- 4- salts
- 5- glass of bottle, Gauze

There are four experiments performed per week:

- 1- Total soluble solid (TSS %)
- 2-pH
- 3- Titrable acidity T.A %
- 4- Ethanol concentration EOH %

Tools and equipments that used to produce the vinegar and alcohol in experiences are:

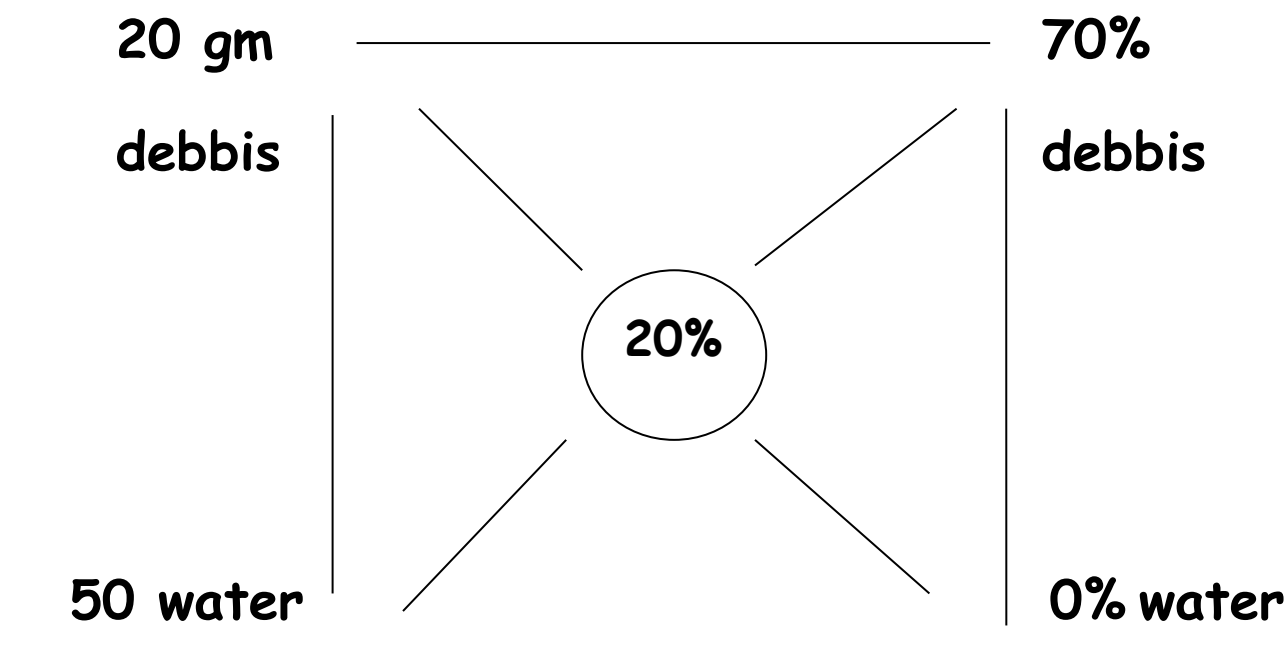
- 1.Refractometer.
- 2.pH meter or pH paper.

3. titration process.

4. Distillation Process.

*TSS% :

To prepare the alcoholic solutions from sugary solid substances have concentration less than 100% or 100% use a person square method, Then use refractometer apparatus to check of the concentration.



70 gm

For example of the application the Person square
To prepare the sugary solution its concentration
=20% & its quantity =3000 grams :

We need water and a sugary substance

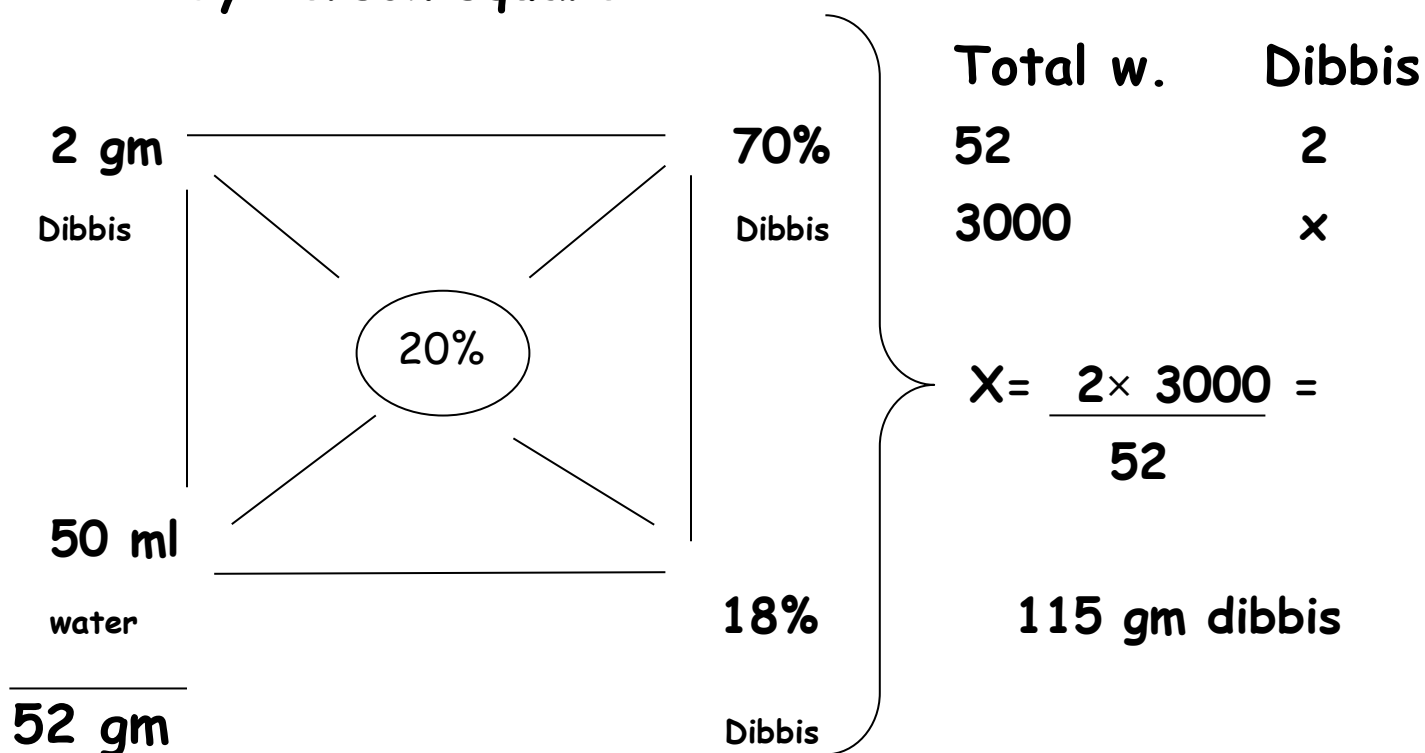
<u>Total Weight</u>	<u>Dibbis</u>
70 gm	20 gm
3000 gm	x

$$X = \frac{3000 \times 20}{70} = 857 \text{ gm / dibbis}$$

$$3000 - 857 = 2143 \text{ ml (water)}$$

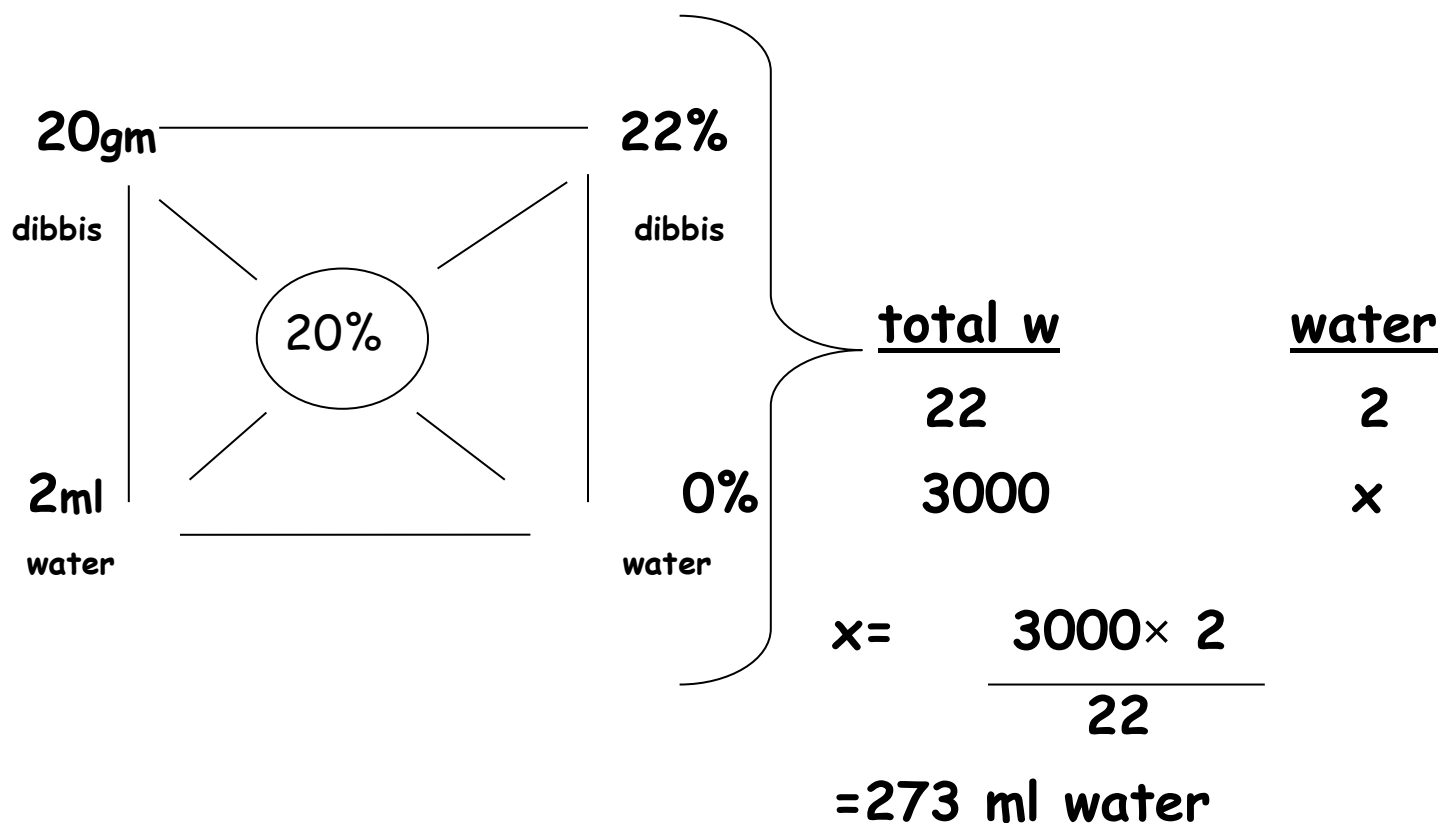
If wanted the solution its concentration 20% of sugar but after examination found its concentration 18%, must be Corrected this Wrong concentration As follows:-

by Person square



So, The Correction must increase the concentration to 20 by adding 115 gm of dibbis

But in the case the concentration of sugar in solution was Higher than required e.g. 22%, must be Corrected this Wrong concentration As follows:-



So, The Correction must decrease the concentration to 20 by adding 273 ml of water.

***pH measure:**

pH paper is a simple & easy but limited accuracy and is not suitable for colored solutions therefore prefer to use measuring devices.

***Titrable acidity :**

To measure percentage of ionized & non-ionized acids ,unlike the pH reading which expresses the percentage of ionized acids only.

Procedure :

1. put 1ml of sugary solution in flask.
- 2.add to flask 19 ml of D.W.
3. add 12 drop of phenolphthalein Reagent.

Calibration with a mixture of NaOH until pink appears; Here, we must stop the titration process and read the volume then calculate the percentage of T.A % as following:

$$\text{T.A. \%} = \frac{\text{ml NaOH} \times \text{Normality of NaOH} \times \text{m.equivalent w.t of acetic acid}}{\text{w.t of sample} \times \text{total titrable volume}} \times 100$$

The volume of the base that used = mk naoh

Normality of naoh

m. equivalent w.t.of acetic acid = 0.06005

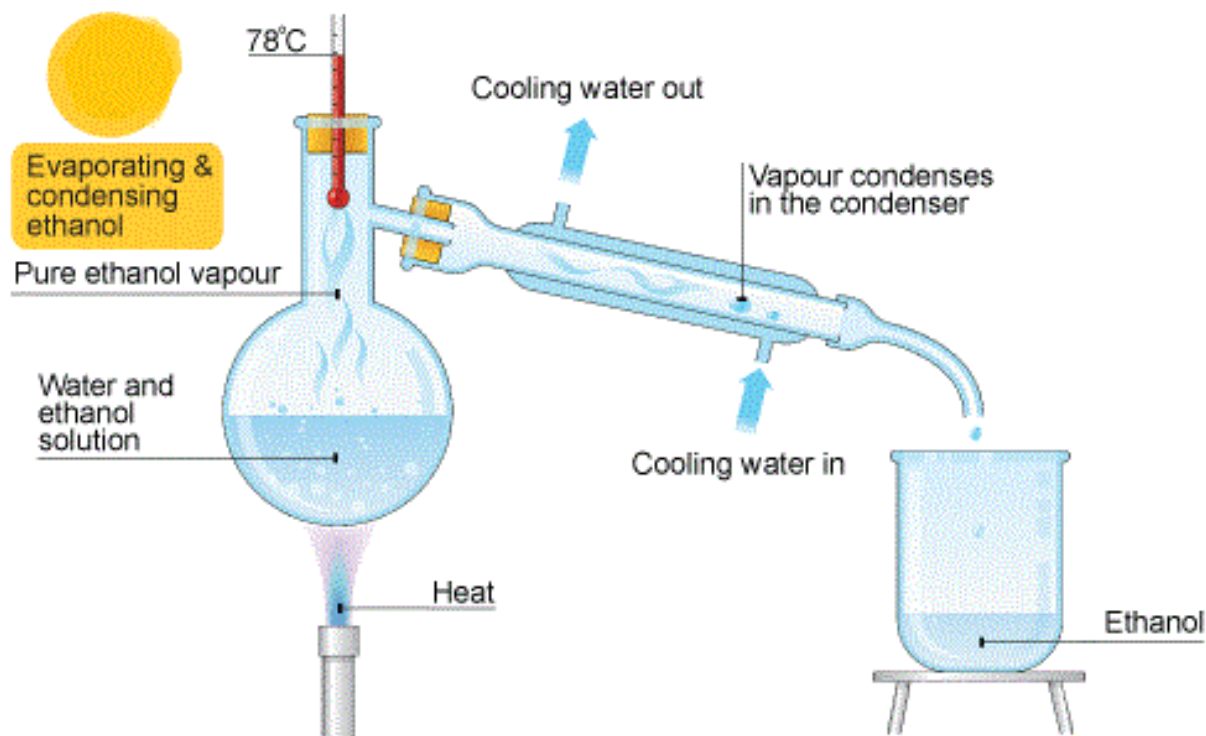
w.t. of sample = 1 gm

total titrable volume = (1 gm + 19 ml D.W + 12 drop)

$$\text{T.A\%} = \frac{? \times 0.1 \times 0.06005}{1 \times 20} \times 100$$

*Ethanol concentration (EOH %) :

Detection of ethanol by physical methods such as : (boiling point →
Distillation method → estimation of ethanol by pycnometer).



Procedure:

- 1- put such as 50 ml from sample (sugary solution) in distillation flask .
- 2- Connect the rest of glass tools with Flask for the completion the distillation process by boiling.
- 3- The resulting From the distillation process collected in pycnometer (its volume ranges between 15-25 ml) .
- 4- Determine the specific gravity of ethanol concentration at every-time after distillation process of the sample, calculate the specific gravity by the following formula :

$$\text{Specific gravity} = \frac{\text{weight of pycnometer with the sample} - \text{its weight without sample (empty)}}{\text{Weight of pycnometer with water} - \text{its weight without water(empty)}}$$

W= wt. of pycnometer empty.

W1= wt of pycnometer with D.W

W2= wt. of pycnometer with sample .

- 5- compare the results of specific gravity each week in Lab. With the previous concentrations of sugary solution sample.

Shake the bottles a week before taking the sample and recording the readings.

week	TSS%	PH	T.A %	EOH %	
0					
1					
2					Type of fermentation
3					
4					
5	Type of fermentation				
6				

Other requirements are added to the mixture solution:

salts for example: Potassium chloride , ammonium phosphate, ammonium sulfate (0.1-0.5 %).