International Journal of scientific research and management (IJSRM)
||Volume||4||Issue||08||Pages||4550-4561||2016||
Website: www.ijsrm.in ISSN (e): 2321-3418

Fermentability Of *Saccharomyces cerevisiae* (Baker's Yeast) With Fruit Juices & Carbohydrate substrates

[1]Dr.Vijaykumar Biradar, [2]Ms. Ashashree Inamdar and [3]Dr. M.S.Patil
HOD & Director, PG Studies & Research Centre in Biotechnology, Karnatak College, Bidar-585 401, Karnataka state, Email ID: drbiradarva@gmail.com
[2]Asst. Professor in Biotechnology, Department of Biotechnology, Karnatak College, Bidar-585 401, Karnataka state, Email ID: ashashree.inamdar@gmail.com
[3]HOD, Department of Botany, Karnatak College, Bidar-585 401, Karnataka state, Email ID: mspkcb1984@gmail.com

ABSTRACT:

Present study was carried out to know the fermentability of unicellular Baker's yeast (Saccharomyces cerevisae) with sugar samples & various juices. The standard 20% sugar solutions (such as Glucose, Fructose, Sucrose, Lactose & Starch) were prepared as control. Various plant juices (Sugar cane, Black grapes, Pine apple, Green grapes & Orange.) of 100ml each were prepared by crushing & filtering. Each juice was diluted by adding 400ml of sterilized water. Each of juice sample & sugar solution was inoculated with 8 gms of wet yeast (w/v) under aseptic conditions & other nutrients like ammonium sulphate $(NH_4)_2SO_4$ were added & the pH is adjusted to 4-5 for proper growth of yeast. All samples were maintained in the incubator at 32°C for 72hrs & were checked for fermentation based on rate of frothing. Based on level of frothing, the amount of ethanol produced & the potential ability of yeast fermentation were determined. It was found that frothing level of samples is not related to quantity production of ethanol. The pH of all fermented samples except in orange juice gradually reduced until 72 hrs of fermentation. Out of all fermented juices, Sugar cane is found to be highest ethanol producing feed stock due to Sucrose content. This is evidenced by results with standard carbohydrate solutions. The glucose is found to be second preferred feed stock for yeast activity followed by Fructose & least with Lactose. The starch has no role in fermentation by yeast. Therefore, composition of fruit juices especially with respect to Sucrose & Glucose play an important role in reduction of inhibitors, & quantity production of ethanol by yeast.

KEYWORDS: *Fermentation, Saccharomyces cerevisae (yeast), Frothing, Ethanol, Enzymes*

1. Introduction:

Fermentations are carried out in the absence of oxygen by strictly anaerobic (Genus: Clostridium) or facultatively anaerobic (lactic acid bacteria & yeasts) microorganisms. The strict anaerobes usually lack catalase activity, & the little peroxidase activity that they may possess, which cannot remove the highly toxic hydrogen peroxide as fast as it is produced during aerobic growth. In certain instances, fermentations utilizing facultative anaerobes, such as the yeasts, employ aeration during inoculums build-up to increase cell numbers

before anaerobic fermentation conditions are imposed. Thus, the rate & amount of cell growth is usually greater for aerobic conditions of growth than for anaerobic.

Microorganisms growing anaerobically recover less energy per unit of carbon substrate utilized than do aerobes. Also, there is tendency in these fermentations for carbon substrates to undergo only partial decomposition so that various organic acids, organic amines, & so forth accumulate in the growth medium, obviously, these products can present problems in pH maintenance of the fermentation. Thus, the incomplete utilization of substrate & low cellular-energy yields for these fermentations often require that much carbon substrate be decomposed for the growth & maintenance of the microbial cells. This phenomenon however, can be beneficial to fermentation yields. Relatively greater passage of substrate carbon through the metabolic sequences of the organism causes a resultant conversion of large amounts of substrate carbon to fermentation product.

Fermentations are intriguing for commercial use (**Susana et. al., 2006)**, because they do not require the expense of large volumes of sterile air or the expense of energy input into the fermentation in the form of vigorous impeller action. Various anaerobic microorganisms possess the ability to degrade cellulose **(Susana & Leschine, 1995).** The commercial production of acetone & butanol by species of the genus *clostridium* will be a described as representative of an anaerobic fermentation utilizing a strictly anaerobic bacterium. Brewing, industrial alcohol, & lactic acid fermentations are included as examples of industrial anaerobic fermentations which are more tolerant of oxygen. The present study is carried out to find-out amount of ethanol production with various substances by yeasts.

1.1 Yeast: It is a member of the fungus family & is a single–celled fungi of which there are about 160 different species. Baker's yeast belongs to the *Saccharomyces cerevisiae* species. The two forms of Baker's yeast are fresh yeast & dry yeast.

(1) Fresh yeast is ivory colored with a yellowish hue & is soft & moist & should easily crumble. Make sure it fresh smells & there are no dark or dried places on the yeast. It is mainly used by professionals as it is highly perishable & must be used within a short time of opening. For longer term storage it can be frozen. Compressed yeast contains about 70% moisture. It needs to be proofed before using & should have a pleasant yeasty smell & be foamy.

(2) Dry yeast is fresh compressed yeast that has been pressed & dried until the moisture contents is only about 8% which makes the yeast dormant. The granules only become active again when mixed with a warm liquid. The advantage of dry yeast is it has a much longer shelf life the fresh yeast & does not need to be refrigerated.

The most well known & commercially significant yeasts are the related species & strains of *Saccharomyces cerevisiae (***Maheshwari et al, 2010)**.These organisms have long been utilized to ferment the sugar of rice, wheat, barley & corn to produce alcoholic beverages & in the baking industry to expand or raise dough. *Saccharomyces cerevisiae* is commonly used as baker's yeast & for other fermentations. Yeast is often taken as a vitamin supplement because it is 50% protein & is a rich source of B vitamins, niacin & folic acid.

The fermentation of wine is initiated by naturally occurring yeasts present in the vineyards. Many wineries still use nature strains, however many use modern methods of strain maintenance & isolation. The bubble in sparkling wines is by trapped carbon dioxide **(Ronald, 2014)**, the result of yeast fermenting sugars in the grape juice. One yeast cell can ferment approximately its own weight of glucose per hour. Under optimal conditions, it can produce up to 18 percent (v) ethanol with 15 to 16 percent being the norm. The sulphur dioxide present in commercially produced wine is actually added just after the grapes are crushed to kill the naturally present bacteria, molds & yeasts.

1.1.1 Y**east growth:**

Yeast requires three things to grow such as moisture, food & warmth. To activate yeast the first step is called proofing by which, one has to make sure that whether a yeast is alive & active. This is accomplished by mixing the yeast in a warm liquid, furher activity of yeast is acheved by adding food. Its favourite food is sugar, simple sugars to be precise (glucose & fructose). Some recipes call for adding

DOI: 10.18535/ijsrm/v4i8.07

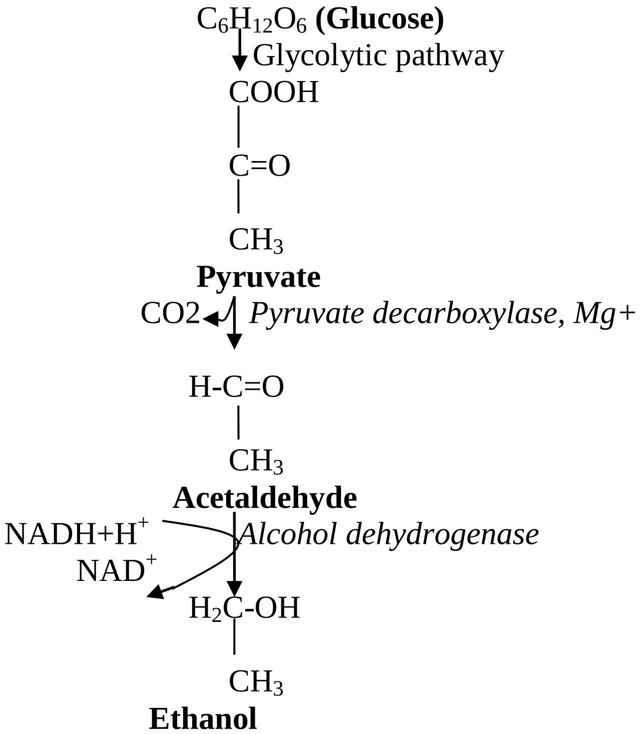
granulated white sugar which the yeast will break down into its simpler form. But is some bread recipes where sugar is no used, flour can be added to the warm liquid & the yeast will break down some of the starch in the flour to a simple sugar.

1.1.2 Uses of Baker's yeast:

The useful physiological properties of yeast have led their use in the field of biotechnology. Fermentation of sugars by yeast is the oldest & largest application of this technology. Baker's yeast is used for bread production. Yeast is also used for wine fermentation. Yeast is also one of the most widely used model organisms for genetics & cell biology.

1.2 Ethanol fermentation:

Yeasts (*Saccharomyces cervisiae*) ferment glucose to ethanol via glycolytic pathway (Jain et. al. 2005). In fermentation, the glucose is converted to pyruvate by series of reactions and each reaction is controlled by specific enzymes. The pyruvate is then decarboxylated to acetaldehyde by an enzyme, pyruvate decarboxylase, which is then reduced to ethanol by alcohol dehydrogenase in presence of electron donor NADH.



The present study was carried out to know the fermentability of unicellular Baker's yeast (*Saccharomyces cerevisae*) with sugar samples & various juices, to study the potential ability of yeast to produce alcohol with various substrates, to determine the fermentation time with various juices & carbohydrates, to study the various parameters that affects on the activity of yeast in various diet, and to compare the rate of alcohol production with various fruit juices & compare with std. carbohydrate solutions.

2. Materials & Methods:

2.1 Preaparation of pure (standard) carbohydrate solutions (as control):

Pure AR grade Glucose, Fructose, Sucrose, Lactose & Starch were purchased from the Sai chemicals disributor, Bidar. Standard carbohydrate solutions were prepared by separate dissolving of 80gms of sugar into 400ml of sterilized water.

DOI: 10.18535/ijsrm/v4i8.07

2.2 Saccharomyces cerevisiae (Dry Baker's yeast):

Pure baker's yeast cells (dry powder) preserved in deepfreezer condition was purchased from super market of Bidar on the day of inoculation into juices & controls.

2.3 Preparation of juices:

The materials colleced forextracting juices were Sugarcane (*Saccharum officinarium*), Black grapes (*Vitis vinifera*), Pineapple (*Ananas comosus*), Green grapes (*Vitis vinifera*) & Orange (*Citrus sinensis*). The fruits were washed, peeled & made the fruits into small pieces. These small pieces were ground separately with the help of mixer grinder to get the extract of 100ml & final volume of juice made up to 400ml by adding sterilized water.

2.4 Preparation of subsrates & Inoculation of yeast for fermentation:

Yeast cells inoculated (80gm/substrate) in fruit juices & standard carbohydrate solutions under aseptic conditions in the laminar air flow cabinet. Additional nutrient such as ammonium sulphate was added for better growth of yeast cells & pH of each sample is checked & adjusted to 4-5. The samples were kept in bacteriological incubator at a temperature of 32°C. Samples were checked every minute for observing frothing level & left undisturbed for 72 hours for complete fermentation. After 72 hour, the samples were filtered & taken for fractional distillation process.

The fractional apparatus consist of a round bottom flask in which the filtered sample is taken. The flask is connected to a condenser & the condenser is connected to a collecting flask in which the pure ethyl alcohol is collected. The round bottom flask is heated at 80°C so that the alcohol is vaporized & condensed by condenser & collected. Distilled sample of above 250ml is taken in a measuring cylinder. The temperature (°F) & the water content (%) of distilled samples were measured with the help of thermometer & hydrometer respectively. The readings of temperature & water content were used for the calculation of percentage of ethanol using standard spirit tables 1 to 4.

TEMPERATURE (80^0F)

Indication	Pe % over proof	Indication	P % over proof	Indication	Pe % over proof	Indication	% over proof	Indication	P % over proof	Indication	P % over proof
40.0	19.7	50.0	6.1	60.0	8.9	70.0	26.4	80.0	48.0	90.0	77.2
.2	19.4	.2	5.8	.2	9.2	.2	26.8	.2	48.6	.2	77.8
.4	19.2	.4	5.6	.4	9.5	.4	27.2	.4	49.1	.4	78.4
.6	18.9	.6	5.3	.6	9.8	.6	27.6	.6	49.6	.6	78.9
.8	18.7	.8	5.0	.8	10.2	.8	27.9	.8	50.2	.8	79.5
41.0	18.4	51.0	4.7	61.0	10.5	71.0	28.3	81.0	50.7	91.0	80.0
.2	18.1	.2	4.4	.2	10.8	.2	28.7	.2	51.2	.2	80.5
.4	17.9	.4	4.2	.4	11.1	.4	29.1	.4	51.8	.4	81.1
.6	17.6	.6	3.9	.6	11.4	.6	29.5	.6	52.3	.6	81.6

.8	17.3	.8	3.6	.8	11.8	.8	29.8	.8	52.9	.8	82.2
42.0	17.1	52.0	3.3	62.0	12.1	72.0	30.2	82.0	53.5	92.0	82.7
.2	16.8	.2	3.0	.2	12.4	.2	3.6	.2	54.1	.2	83.3
.4	16.5	.4	2.7	.4	12.8	.4	31.0	.4	54.6	.4	83.8
.6	16.3	.6	2.5	.6	13.1	.6	31.4	.6	55.2	.6	84.3
.8	16.0	.8	2.2	.8	13.5	.8	31.8	.8	55.7	.8	84.8
43.0	15.8	53.0	1.9	63.0	13.8	73.0	32.2	83.0	56.3	93.0	85.3
.2	15.5	.2	1.6	.2	14.1	.2	32.6	.2	56.7	.2	85.8
.4	15.2	.4	1.3	.4	14.5	.4	33.0	.4	57.4	.4	86.3
.6	14.9	.6	1.0	.6	14.9	.6	33.4	.6	58.0	.6	86.8
.8	14.6	.8	0.7	.8	15.2	.8	33.8	.8	58.6	.8	87.3
44.0	14.4	54.0	0.4	64.0	15.6	74.0	34.2	84.0	59.2	94.0	87.9
.2	14.1	.2	0.1	.2	15.9	.2	34.7	.2	59.7	.2	88.4
.4	13.8	.4	0.2	.4	16.3	.4	35.1	.4	60.3	.4	88.9
.6	13.6	.6	0.2	.6	16.6	.6	35.5	.6	60.9	.6	89.4
.8	13.3	.8	0.8	.8	16.9	.8	35.9	.8	61.5	.8	89.9
45.0	13.	55.0	1.1	65.0	17.3	75.0	36.3	85.0	62.1	95.0	90.4
.2	12.8	.2	1.4	.2	17.6	.2	36.8	.2	62.7	.2	90.9
.4	12.5	.4	1.7	.4	18.0	.4	37.2	.4	63.3	.4	91.4
.6	12.2	.6	2.0	.6	18.3	.6	37.6	.6	63.9	.6	91.9
.8	11.9	.8	2.3	.8	18.7	.8	38.1	.8	64.4	.8	92.5
46.0	11.6	56.0	2.6	66.0	19.0	76.0	38.5	86.0	65.0	96.0	93.0
.2	11.4	.2	3.0	.2	19.4	.2	39.0	.2	65.6	.2	93.5
.4	11.1	.4	3.3	.4	19.7	.4	39.5	.4	66.2	.4	94.0
.6	10.8	.6	3.6	.6	20.1	.6	39.9	.6	66.8	.6	94.5
.8	10.5	.8	3.9	.8	20.5	.8	40.3	.8	67.4	.8	94.9
47.0	10.2	57.0	4.2	67.0	20.8	77.0	40.8	87.0	68.0	97.0	95.4
.2	10.0	.2	4.5	.2	21.2	.2	41.2	.2	68.6	.2	95.9
.4	9.7	.4	4.8	.4	21.5	.4	41.7	.4	69.3	.4	96.4
.6	9.4	.6	5.1	.6	21.9	.6	42.2	.6	69.9	.6	96.9
.8	9.1	.8	5.4	.8	22.3	.8	42.7	.8	70.5	.8	97.4
48.0	8.9	58.0	5.7	68.0	22.6	78.0	43.1	88.0	71.2	98.0	97.8
.2	8.6	.2	6.0	.2	23.0	.2	43.6	.2	71.8	.2	98.3
.4	8.3	.4	6.3	.4	23.4	.4	44.1	.4	72.4	.4	98.8
.6	8.1	.6	6.7	.6	23.7	.6	44.6	.6	73.0	.6	99.3
.8	7.8	.8	7.0	.8	24.1	.8	45.1	.8	73.6	.8	99.8
49.0	7.5	59.0	7.3	69.0	24.5	79.0	45.6	89.0	74.1	99.0	-
.2	7.2	.2	7.6	.2	24.8	.2	46.1	.2	74.7	.2	-
.4	6.9	.4	7.9	.4	25.2	.4	46.6	.4	75.2	.4	-
.6	6.6	.6	8.3	.6	25.6	.6	47.1	.6	75.6	.6	-
.8	6.3	.8	8.6	.8	25.9	.8	47.6	.8	76.7	.8	-
50.0	6.1	60.0	8.9	70.0	26.4	80.0	48.0	90.0	77.2	100.0	-

DOI: 10.18535/ijsrm/v4i8.07

Table-1: Standard spirit table

TEMPERATURE (82^{0}F)

Indication	% over proof	Indication	% over proof	Indication	%over proof	Indication	% over proof	Indication	% over proof	Indication	% over proof
40.0	19.	50.0	5.4	60.0	9.6	70.0	27.1	80.0	48.8	90.0	77.8
.2	18.8	.2	5.1	.2	10.0	.2	27.5	.2	49.3	.2	78.3
.4	18.5	.4	4.9	.4	10.3	.4	27.8	.4	49.9	.4	78.9
.6	18.3	.6	4.6	.6	10.6	.6	28.2	.6	50.4	.6	79.4
.8	18.0	.8	4.3	.8	10.9	.8	28.6	.8	50.9	.8	79.9
41.0	17.7	51.0	4.0	61.0	11.2	71.0	28.9	81.0	51.5	91.0	80.5
.2	17.5	.2	3.7	.2	11.5	.2	29.3	.2	52.0	.2	81.0
.4	17.2	.4	3.5	.4	11.9	.4	29.7	.4	52.6	.4	81.6
.6	16.9	.6	3.2	.6	12.2	.6	30.1	.6	53.1	.6	82.1
.8	16.7	.8	2.9	.8	12.5	.8	30.5	.8	53.7	.8	82.7
42.0	16.4	52.0	2.6	62.0	12.8	72.0	30.9	82.0	54.3	92.0	83.2
.2	16.1	.2	2.3	.2	13.2	.2	31.3	.2	54.8	.2	83.7
.4	15.9	.4	2.0	.4	13.5	.4	31.7	.4	55.4	.4	84.2
.6	15.6	.6	1.7	.6	13.9	.6	32.2	.6	55.9	.6	84.7
.8	15.4	.8	1.5	.8	14.2	.8	32.6	.8	56.5	.8	85.2
43.0	15.1	53.0	1.2	63.0	14.5	73.0	33.0	83.0	57.0	93.0	85.8
.2	14.8	.2	0.9	.2	14.9	.2	33.4	.2	57.6	.2	86.3
.4	14.5	.4	0.6	.4	15.2	.4	33.8	.4	58.2	.4	86.8
.6	14.3	.6	0.3	.6	15.6	.6	34.2	.6	58.8	.6	87.3
.8	14.0	.8	Proof	.8	16.0	.8	34.6	.8	59.3	.8	87.8
44.0	13.7	54.0	0.3	64.0	16.3	74.0	35.0	84.0	59.9	94.0	88.3
.2	13.4	.2	0.6	.2	16.7	.2	35.4	.2	60.5	.2	88.8
.4	13.2	.4	.9	.4	17.0	.4	35.8	.4	61.1	.4	89.3
.6	12.9	.6	1.2	.6	17.3	.6	36.3	.6	61.6	.6	89.8
.8	12.6	.8	1.5	.8	17.7	.8	36.7	.8	62.2	.8	90.3
45.0	12.4	55.0	1.8	65.0	18.0	75.0	37.1	85.0	62.8	95.0	90.8
.2	12.1	.2	2.1	.2	18.4	.2	37.5	.2	63.4	.2	91.3
.4	11.8	.4	2.4	.4	18.7	.4	38.0	.4	64.0	.4	91.8
.6	11.5	.6	2.7	.6	19.1	.6	38.4	.6	64.6	.6	92.3
.8	11.2	.8	3.1	.8	19.4	.8	39.8	.8	65.1	.8	92.9
46.0	11.0	56.0	3.4	66.0	19.8	76.0	39.3	86.0	65.7	96.0	93.4
.2	10.7	.2	3.7	.2	20.1	.2	39.7	.2	66.3	.2	93.9
.4	10.4	.4	4.0	.4	20.5	.4	40.2	.4	66.9	.4	94.4
.6	10.1	.6	4.3	.6	20.9	.6	40.6	.6	67.5	.6	94.8
.8	9.8	.8	4.6	.8	21.2	.8	41.1	.8	68.1	.8	95.3
47.0	9.6	57.0	4.9	67.0	21.6	77.0	41.5	87.0	68.7	97.0	95.8
.2	9.3	.2	5.2	.2	21.9	.2	42.0	.2	69.3	.2	96.3
.4	9.0	.4	5.5	.4	22.3	.4	42.5	.4	69.9	.4	96.8
.6	8.7	.6	5.8	.6	22.7	.6	43.0	.6	70.5	.6	97.2
.8	8.5	.8	6.1	.8	23.0	.8	43.4	.8	71.2	.8	97.7
48.0	8.2	58.0	6.4	68.0	23.4	78.0	43.9	88.0	71.8	98.0	98.2
.2	7.9	.2	6.8	.2	23.8	.2	44.4	.2	72.4	.2	98.7
.4	7.6	.4	7.1	.4	24.1	.4	44.9	.4	73.0	.4	98.1
.6	7.4	.6	7.4	.6	24.5	.6	45.3	.6	73.6	.6	99.6
.8	7.1	.8	7.7	.8	24.9	.8	45.8	.8	74.2	.8	100.0
49.0	6.8	59.0	8.0	69.0	25.2	79.0	46.3	89.0	74.8	99.0	-
.2	6.5	.2	8.4	.2	25.6	.2	46.8	.2	75.4	.2	-
.4	6.3	.4	8.7	.4	26.0	.4	47.3	.4	76.0	.4	-
.6	6.0	.6	9.0	.6	26.4	.6	47.8	.6	76.6	.6	-
.8	5.7	.8	9.3	.8	26.7	.8	48.3	.8	77.2	.8	-
50.0	5.4	60.0	9.6	70.0	27.1	80.0	48.8	90.0	77.8	100.0	-

DOI: 10.18535/ijsrm/v4i8.07

Table-2: Standard spirit table

TEMPERATURE (83^0F)

Indication	% over proof	Indication	% over proof	Indication	%over proof	Indication	%over proof	Indication	%over proof	Indication	%over proof
40.0	18.7	50.0	5.1	60.0	10.0	70.0	27.5	80.0	49.2	90.0	78.1
.2	18.5	.2	4.8	.2	10.3	.2	27.9	.2	49.7	.2	78.6
.4	18.2	.4	4.5	.4	10.6	.4	28.3	.4	50.2	.4	79.1
.6	17.9	.6	4.2	.6	11.0	.6	28.7	.6	50.8	.6	79.7
.8	17.7	.8	4.0	.8	11.3	.8	29.1	.8	51.3	.8	80.2
41.0	17.4	51.0	3.7	61.0	11.6	71.0	29.5	81.0	51.8	91.0	80.7
.2	17.1	.2	3.4	.2	11.9	.2	29.9	.2	52.4	.2	81.3
.4	16.9	.4	3.1	.4	12.2	.4	30.3	.4		.4	81.8
.6	16.6	.6	2.8	.6	12.6	.6	30.7	.6		.6	82.4
.8	16.3	.8	2.5	.8	12.9	.8	31.1	.8	54.1	.8	82.9
42.0	16.1	52.0	2.3	62.0	13.2	72.0	31.5	82.0	54.6	92.0	83.5
.2	15.8	.2	2.0	.2	13.6	.2	31.9	.2	55.2	.2	84.0
.4	15.6	.4	1.7	.4	13.9	.4	32.3	.4	55.7	.4	84.5
.6	15.3	.6	1.4	.6	14.2	.6	32.6	.6	56.3	.6	85.0
.8	15.0	.8	1.1	.8	14.6	.8	33.0	.8	56.9	.8	85.5
43.0	14.8	53.0	0.8	63.0	14.9	73.0	33.4	83.0	57.4	93.0	86.0
.2	14.5	.2	0.5	.2	15.3	.2	33.8	.2	58.0	.2	86.5
.4	14.2	.4	0.2	.4	15.6	.4	34.2	.4	58.5	.4	87.0
.6	13.9	.6	0.1	.6	16.0	.6	34.6	.6	59.1	.6	87.5
.8	13.6	.8	0.4	.8	16.3	.8	35.0	.8	59.7	.8	88.0
44.0	13.4	54.0	0.7	64.0	16.7	74.0	35.4	84.0	60.3	94.0	88.5
.2	13.1	.2	1.0	.2	17.0	.2	35.8	.2	60.9	.2	89.0
.4	12.8	.4	1.3	.4	17.4	.4	36.2	.4	61.4	.4	89.5
.6	12.6	.6	1.6	.6	17.7	.6	36.6	.6	62.0	.6	90.0
.8	12.3	.8	1.9	.8	18.1	.8	37.1	.8	62.6	.8	90.5
45.0	12.0	55.0	2.2	65.0	18.4	75.0	37.8	85.0	63.2	95.0	91.0
.2	11.8	.2	2.5	.2	18.8	.2	37.9	.2	63.8	.2	91.5
.4	11.5	.4	2.8	.4	19.1	.4	38.3	.4	64.3	.4	92.0
.6	11.2	.6	3.1	.6	19.5	.6	38.8	.6	64.9	.6	92.6
.8	10.9	.8	3.4	.8	19.8	.8		.8	65.5	.8	93.1
46.0	10.6	56.0	3.7	66.0	20.2	76.0	39.6	86.0	66.1	96.0	93.6
.2	10.3	.2	4.0	.2	20.5	.2	40.1	.2	66.7	.2	94.1
.4	10.1	.4	4.4	.4	20.9	.4	40.6	.4	67.3	.4	94.5
.6	9.8	.6	4.7	.6	21.2	.6	41.0	.6	67.8	.6	95.0
.8	9.5	.8	5.0	.8	21.6	.8	41.5	.8	68.4	.8	95.5
47.0	9.2	57.0	5.3	67.0	22.0	77.0	41.9	87.0	69.0	97.0	96.0
.2	9.0	.2	5.6	.2	22.3	.2	42.4	.2	69.6	.2	96.5
.4	8.7	.4	5.9	.4	22.7	.4	42.9	.4	70.3	.4	97.0
.6	8.4	.6	6.2	.6	23.0	.6	43.3	.6	70.9	.6	97.4
.8	8.1	.8	6.5	.8	23.4	.8	43.8	.8	71.5	.8	97.9
48.0	7.8	58.0	6.8	68.0	23.8	78.0	44.3	88.0	72.1	98.0	98.4
.2	7.6	.2	7.1	.2	24.1	.2	44.8	.2	72.7	.2	98.8
.4	7.3	.4	7.5	.4	24.5	.4	44.2	.4	73.3	.4	99.3
.6	7.0	.6	7.8	.6	24.9	.6	45.7	.6	73.9	.6	99.8
.8	6.8	.8	8.1	.8	25.2	.8	46.2	.8	74.5	.8	-
49.0	6.5	59.0	8.4	69.0	25.6	79.0	46.7	89.0	75.1	99.0	-
.2	6.2	.2	8.7	.2	26.0	.2	47.2	.2	75.7	.2	-
.4	5.9	.4	9.1	.4	26.4	.4	47.7	.4	76.3	.4	-
.6	5.6	.6	9.4	.6	26.7	.6	48.2	.6	76.9	.6	-
.8	5.4	.8	9.7	.8	27.1	.8	48.7	.8	77.5	.8	-
50.0	5.1	60.0	10.0	70.0	27.5	80.0	49.2	90.0	78.1	100.0	-

DOI: 10.18535/ijsrm/v4i8.07

Table-3: Standard spirit tabl

TEMPERATURE (84^0F)

Indication	% over proof	Indication	% over proof	Indication	% over proof	Indication	% over proof	Indication	% over proof	Indication	% over proof
40.0	18.4	50.0	4.7	60.0	10.4	70.0	27.9	80.0	49.6	90.0	78.3
.2	18.1	.2	4.5	.2	10.7	.2	28.2	.2	50.1	.2	78.9
.4	17.9	.4	4.2	.4	11.0	.4	28.6	.4	50.6	.4	79.4
.6	17.6	.6	3.9	.6	11.3	.6	29.0	.6	51.2	.6	79.9
.8	17.3	.8	3.6	.8	11.6	.8	29.3	.8	51.7	.8	80.5
41.0	17.1	51.0	3.3	61.0	12.0	71.0	29.7	81.0	52.2	91.0	81.0
.2	16.8	.2	3.0	.2	12.3	.2	30.1	.2	52.8	.2	81.5
.4	16.5	.4	2.8	.4	12.6	.4	30.5	.4	53.3	.4	82.1
.6	16.3	.6	2.5	.6	12.9	.6	30.9	.6	53.9	.6	82.6
.8	16.0	.8	2.2	.8	13.3	.8	31.3	.8	54.5	.8	83.1
42.0	15.7	52.0	1.9	62.0	13.6	72.0	31.7	82.0	55.0	92.0	83.7
.2	15.5	.2	1.6	.2	13.9	.2	32.1	.2	55.6	.2	84.2
.4	15.2	.4	1.3	.4	14.3	.4	32.5	.4	56.1	.4	84.7
.6	15.0	.6	1.0	.6	14.6	.6	32.9	.6	56.7	.6	85.2
.8	14.7	.8	0.8	.8	14.9	.8	33.3	.8	57.2	.8	85.7
43.0	14.5	53.0	0.5	63.0	15.3	73.0	33.7	83.0	57.8	93.0	86.2
.2	14.2	.2	0.2	.2	15.6	.2	34.1	.2	58.4	.2	86.7
.4	13.9	.4	-	.4	16.0	.4	34.6	.4	58.9	.4	87.2
.6	13.6	.6	0.4	.6	16.3	.6	35.0	.6	59.5	.6	87.7
.8	13.3	.8	0.7	.8	16.7	.8	35.4	.8	60.1	.8	88.2
44.0	13.0	54.0	1.0	64.0	17.1	74.0	35.8	84.0	60.6	94.0	88.7
.2	12.8	.2	1.3	.2	17.4	.2	36.2	.2	61.2	.2	89.2
.4	12.5	.4	1.6	.4	17.7	.4	36.6	.4	61.8	.4	89.7
.6	12.2	.6	2.0	.6	18.1	.6	37.0	.6	62.4	.6	90.2
.8	12.0	.8	2.3	.8	18.4	.8	37.5	.8	63.0	.8	90.7
45.0	11.7	55.0	2.6	65.0	18.8	75.0	37.9	85.0	63.5	95.0	91.2
.2	11.4	.2	2.9	.2	19.1	.2	38.3	.2	64.1	.2	91.7
.4	11.1	.4	3.2	.4	19.5	.4	38.7	.4	64.7	.4	92.2
.6	10.8	.6	3.5	.6	19.8	.6	39.2	.6	65.3	.6	92.8
.8	10.6	.8	3.8	.8	20.2	.8	39.6	.8	65.9	.8	93.3
46.0	10.3	56.0	4.1	66.0	20.5	76.0	40.0	86.0	66.4	96.0	93.8
.2	10.0	.2	4.4	.2	20.9	.2	40.5	.2	67.0	.2	94.3
.4	9.7	.4	4.7	.4	21.3	.4	40.9	.4	67.6	.4	94.7
.6	9.4	.6	5.0	.6	21.6	.6	41.4	.6	68.2	.6	95.2
.8	9.2	.8	5.3	.8	22.0	.8	41.8	.8	68.8	.8	95.7
47.0	8.9	57.0	5.7	67.0	22.3	77.0	42.3	87.0	69.4	97.0	96.2
.2	8.6	.2	6.0	.2	22.7	.2	42.7	.2	70.0	.2	96.7
.4	8.3	.4	6.3	.4	23.1	.4	43.2	.4	70.6	.4	97.1
.6	8.1	.6	6.6	.6	23.4	.6	43.7	.6	71.2	.6	97.6
.8	7.8	.8	6.9	.8	23.8	.8	44.2	.8	71.8	.8	98.1
48.0	7.5	58.0	7.2	68.0	24.1	78.0	44.7	88.0	72.4	98.0	98.5
.2	7.2	.2	7.5	.2	24.5	.2	45.2	.2	73.0	.2	99.0
.4	7.0	.4	7.8	.4	24.9	.4	45.6	.4	73.6	.4	99.5
.6	6.7	.6	8.1	.6	25.2	.6	46.1	.6	74.2	.6	99.9
.8	6.4	.8	8.5	.8	25.6	.8	46.6	.8	74.8	.8	-
49.0	6.1	59.0	8.8	69.0	26.0	79.0	47.1	89.0	75.4	99.0	-
.2	5.9	.2	9.1	.2	26.4	.2	47.6	.2	76.0	.2	-
.4	5.6	.4	9.4	.4	26.7	.4	48.1	.4	76.5	.4	-
.6	5.3	.6	9.7	.6	27.1	.6	48.6	.6	77.1	.6	-
.8	5.0	.8	10.1	.8	27.5	.8	49.1	.8	77.7	.8	-
50.0	4.7	60.0	10.4	70.0	27.9	80.0	49.6	90.0	78.3	100.0	-

Table-4: Standard spirit table

2.5 Calculation of percentage of alcohol (from fruit juices & carbohydrate solutions):

Sugarcane: (& others similar calculations were made)

Temperature: 80°F
Hydrometer reading: 93

Under proof reading: 85.3

Percentage of alcohol= 100-85.3=14.7

=14.7 x C.F.

=14.7 x 0.571

=8.39%

Table 5: Calculated percenage of alcohol in juices & pure carbohydrate solutions

Fruit juices & Std.solutions	Percentage of alcohol
Sugarcane	8.39
Black grapes	3.76
Pineapple	0.22
Green grapes	4.39
Orange	3.75
Lactose	0.11
Fructose	3.36
Sucrose	8.17
Glucose	6.37

3. Results & Discussions:

Various parameters observed during the experiment have shown significant variations of alcohol production with compostions of juices as shown in the table-6. pH of all fermented samples except in orange juice, gradually reduced after 72 hours of fermentation. Out of all fermented fruit juices, sugar cane is found to be highest ethanol producing feed stock with yeast fermentation, due to the sucrose content (Figs. 3 & 5). This is evidenced by our results obtained with pure carbohydrates (Figs.4 & 6). The glucose is found to be second preferred feed stock for yeast activity, followed by fructose & least with lactose in the fermentation process (Table 7). The starch has no role in fermentation with yeast. Hence the composition of fruit juices especially with respect to carbohydrate contents like sucrose & glucose play an important role in the production of ethanol yeast activity.

Certain enzymes present in yeast can ferment Carbohydrates resulting in the formation of Ethyl alcohol & liberation of carbon-dioxide. The overall reaction is as follows:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH+2CO_2$$

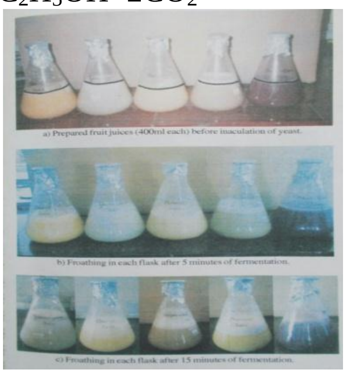


Fig.2: Stages of fermentation process in fruit juices

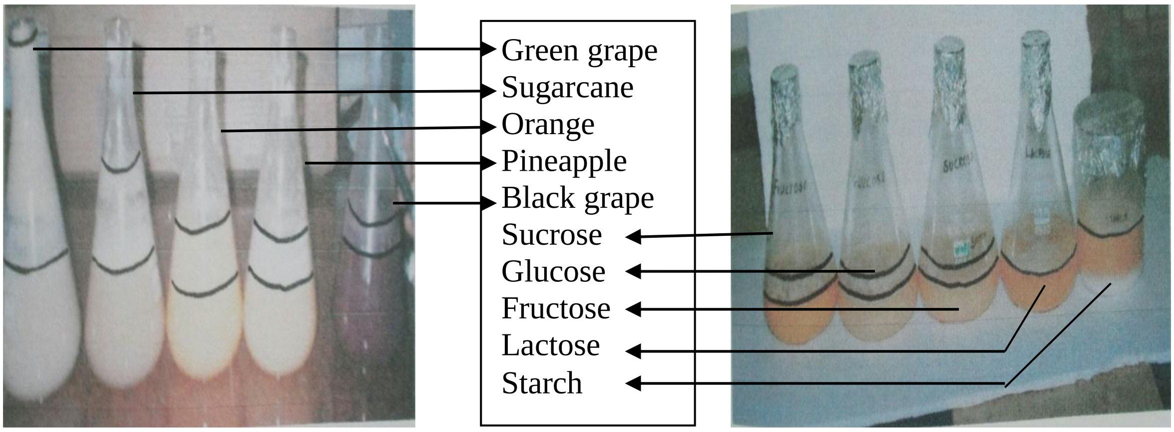


Fig. 3: Final level of froathing in fruit juices. **Fig. 4: Final level of frothing in various pure carbohydrates**

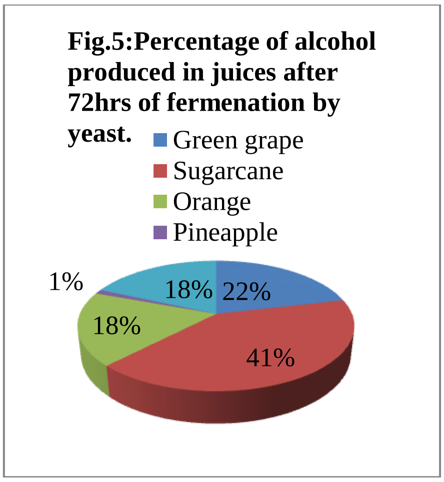
Table 6: Parameters observed & results obtained during & after fermentation of fruit juices.

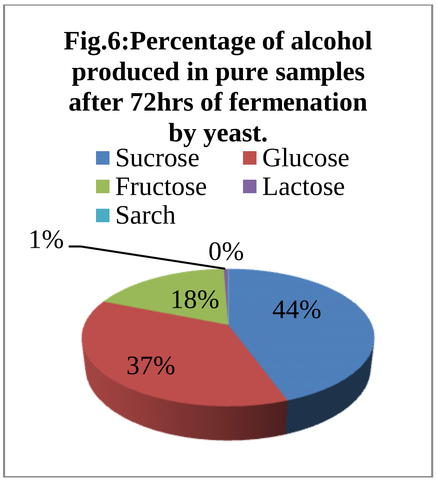
S.no.	Parameters observed	Green grape	Sugar cane	Orange	Pine apple	Black grape
1.	Time of commencement	12:30 pm	12:30 pm	12:30 pm	12:30 pm	12:30pm
2.	pH of juices observed	3.0	5.0	3.5	3.0	3.5
3.	pH of all juices adjusted	4-5	4-5	4-5	4-5	4-5
4.	Temperature of sample before inoculation of yeast	29°C	28°C	28°C	28°C	29°C
5.	Temperature of sample after inoculation	30°C	28°C	30°C	28°C	30°C
6.	Time of inoculation of yeast	2:20 pm	2:20 pm	2:20 pm	2:20 pm	2:20 pm
7.	Frothing initiation time	2:25 pm	2:25 pm	2:25 pm	2:25 pm	2:25 pm
8.	Frothing termination time	2:46 pm	2:46 pm	2:46 pm	2:46 pm	2:46 pm
9.	Frothing level measured	12.5cm	4.5 cm	5 cm	3 cm	2.5 cm
10.	pH of sample observed after 72 hours of fermentation	3.5	3.5	3.5	3.5	4
11.	Total fermentation time	72 hrs	72 hrs	72 hrs	72 hrs	72 hrs
12.	Percentage of alcohol found after 72 hours	4.39%	8.39%	3.76%	6.22%	3.76%
13.	Carbohydrates found in each juice after 72 hours	Glucose Fructose	Sucrose	Glucose Fructose Sucrose	Glucose Fructose Sucrose	Glucose Fructose Sucrose

Table 7: Parameters observed & results obtained during fermentation of pure carbohydrates.

S.no.	Parameters observed	Sucrose	Glucose	Fructose	Lactose	Starch
1.	Time of commencement	12:30pm	12:30pm	12:30 pm	12:30 pm	12:30pm
2.	pH of std. carbohydrate soln. observed	5.5	5	5	5	5
3.	pH of all carbohydrates adjusted	4-5	4-5	4-5	4-5	4-5
4.	Temperature of sample before inoculation of yeast	38°C	35°C	38°C	39°C	45°C
5.	Temperature of sample after addition of yeast	38°C	38°C	38°C	38°C	39°C

6.	Time of inoculation of yeast	1:20 pm	1:20 pm	1:20 pm	1:20 pm	1:20 pm
7.	Frothing initiation time	1:25 pm	1:20 pm	1:25 pm	--	--
8.	Frothing termination time	1:45 pm	1:50 pm	1:55 pm	--	--
9.	Frothing level measured	2.3 cm	2.5cm	3.4 cm	--	--
10.	pH of std. soln. after fermentation	3.5	3.5	3.5	3.5	4
11.	Total fermentation time	72 hrs	72 hrs	72 hrs	72 hrs	72 hrs
12.	Percentage of alcohol found after 72 hours	8.17%	6.73%	3.36%	0.11%	--





This reaction includes different carbohydrates & baker's yeast. The d-Glucose, d-Mannose, Sucrose & Maltose are first hydrolyzed by some specific enzymes present in yeast & thus, resulting mono-saccharides which later ferment into ethanol & carbon dioxide. Carbon dioxide gas bubbles out of the solution into the air leaving a mixture by fractional distillation. Fermentation must be carried out in the absence of air to make alcohol. If air is present, ethanoic acid is made instead of alcohol. Yeast is used in a batch process to make alcohol in beer & wine. An enzyme in yeast acts on the natural sugar in malt (to make beer) & grapes (to make wine). When the alcohol concentration reaches about 10%, the alcohol damages the yeast & fermentation stops. In a batch process the reaction vessel must be emptied & cleaned & then refilled with the new starting materials. A batch process takes more time & is more expensive than a continuous process. Different alcoholic drinks contain different amounts of alcohol. Some people drink alcohol before enjoyment, some drink to excess & some people become addicted to alcohol. The harmful effects (physical & social) of drinking excess alcohol are widespread & reach all parts of society.

In the result starch, since it is a polysaccharide, the yeast may not be assimilated & fermented. However, alcohol fermentation of starch was reported (Yoshioshi et.al. 1997) by the recombinant yeast.Where as other carbohydrates are assimilated & fermented to different ratios of subsrates for ethanol production. But irrespective of their molecular weight the sucrose is found to be preferred as feed stock among monosaccharides & disaccharides. In the present data sugar cane has 16% of sucrose & expected ethanol percentage is also same but estimated ethanol is lesser than the percentage of sucrose. Less ethanol production is due to more than about 9% inhibited the activity of yeast by some inhibitors. It was reported by Lisbeth & Barbel (1996) that, microorganisms produce ethanol with a high yield from all sugars present (hexoses as well as pentoses) and for high ethanol productivity, composition of substrate can withstand potential inhibitors, and therefore the fermentation performance will consequently vary. The inhibition may be partly overcome by the removal of inhibitors, i.e., detoxification.

4. Conclusion:

It was found that frothing level released by fermentation juices is not related to quantity production of ethanol. The pH of all fermented juices except in orange gradually reduced until 72 hours of fermentation. Out of all fermented juices, sugar cane juice was found to be highest ethonol produccing feed stock due to sucrose content as it is more prefered by yeast, because this result was evidenced by our control results. The glucose was found to be second prefered feed stock for yeast activity & ethanol production, followed by fructose & least with lactose. The starch has no role in fermentation by yeast. Therefore, composition of fruit juices especially wih respect to sucrose & glucose play important role in reduction of inhibitors, & quantity production of ethanol by yeast.

5. Acknowledgement:

In retrospect, everyone has to be thanked but some have to be singled out as their help ha been more than just encouragement

- We express our extreme gratiude to our Principal, Dr. B.S. Biradar, Karnatak College, Bidar, for providing laboratory facilities during this work.
- We also heartly thankful to The Director, Distillary Industry, Anadur, Bidar, for his cooperation & helping hand in our work

6. References:

1. Byers B., 1981. Multiple roles of the spindle pole bodies in the life cycle of *Saccharomyces cerevisiae*. In “Alfred Benzon Symposium 16, Molecular Genetics of Yeast” (D. von Wettstein, J. Frisis, M. Kielland-Brandt, & A. Stenderup. Eds.), Munksgaard. Copenhagen (in press).
2. Maheshwari, DK., Dubey, RC. & Saravanathu, R., 2010. Industrial exploitation of microorganisms. IBN 978-93-80026-53-4.
3. Fincham JRS & Day PR., 1971. Fungal Geneics, pp. 402. Blackwell Scientific Publ.
4. Hartwell LH., 1974. Saccharomyces cerevisiae cell cycle. Bacteriol. Rev. 38, 164-198.
5. Jain JL., Jain S. & Jain N., 2005. “Fundamentals of Biochemistry”, S.Chand & Company Ltd (5th Edn), New Delhi.
6. Lisbeh O. & Barbel HH., 1996. Fermenation of lignocellulosic hydrolysates for ethanol production. Enzyme and Microbial Technology 18 (5), 312-331.
7. Ronald SJ., 2014. Wine science, principles & applications. ISBN: 978-0-12-381468-5. Fourth edition.
8. Singh RP., 2009. Applied Microbiology. ISBN 81-272-3171-1
9. Susan BL., 1995. Cellulose degradation in anaerobic environments. Department of Microbiology, University of Massachusetts, Amherst, Massachusetts (1995)01003-5720.Annu.Rev.49:399-426.
10. Susana BL., Rodríguez C. & Sanroman MA., 2006. Application of solid-state fermentation to food industry- A review. Journal of Food Engineering. Volume 76, Issue 3, October 2006, Pages 291–302.
11. Yoshitoshi N., Fumihisa K., Makoto O & Tatsuro S., 1997. Alcohol fermentation of starch by geneic recombinant yeast having glucoamylase acivity. Biotechnology & Bioengineering, 53(1), 21-25.