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Fermentation Process for Manufacturing of Wine from Emblica officinalis fruits

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Abstract—**Amla fruits can be used as a valuable ingredient for the production of an amla wine with all the important properties of wine having medicinal characteristics of amla fruits. A fermenting strain of *Saccharomyces cerevisiae* was utilized for alcoholic fermentation using sugarcane molasses. Temperature is one of the major constraints that determine the ethanol production. The fermentation process was carried out at 25, 30, 35 and 40°C with 20% initial sugar concentration. It was concluded that an increase in alcohol concentration, productivity as well as efficiency with an increase in pH (4.0-5.0) and it was also found that this optimum pH range was suitable for *S. cerevisiae* strain. The fermentation process using *S. cerevisiae* under optimized conditions i.e. pH 6, sugar concentration 20% and temperature 30°C revealed an increase in ethanol production i.e. 8.9 % (v/v) with good fermentation efficiency.**

Index Terms— **Amla (*Emblica officinalis)*, Wine, Fermentation, S. cerevisiae**

I. INTRODUCTION

Amla/Aonla is also known as Indian gooseberry *(Emblica officinalis Gaertn)* is indigenous to Indian subcontinent and a crop of commercial significance. In the world, in area and production of this crop; India ranks first. In nature, growing trees are found in different parts of the world like Sri Lanka, Cuba, Puerto Rico, USA, Iran, Iraq, Pakistan Thailand, China, Malaysia, Vietnam, Japan, Philippines, Panama, Trinidad and Bhutan except in India. Production of good quality wines is the amla/aonla (Emblica syn. Phyllanthus emblica), is an important minor fruit and a crop of commercial significance. It is quite hardy, prolific bearer and is highly remunerative even without much care. It belongs to the family Euphorblacea and is known as amla, amlaki, amali, ambala, amalakamu and nelli in different parts of India. Phyllanthus emblica is mentioned as an evergreen tree even though its leaves are deciduous. The varieties Banarasi and Chakaiya are well known for their large fruit size and are recommended for commercial cultivation. The fruits may be stored in cold storage for 7-8 days at 32-35 ^{0}F. Amla is rare example of an edible material, which is rich in tannis as well as ascorbic acid. Glucose fructose, sucrose and myo-inisitol are the main constituents of amala fruit. The fruit contain many polyphenolic substances, which has antioxidant property and good radical scavenging activity. The fruit also contain alkaloids (Phyllantidine and phyllantine), auxines and minerals. About 500-700mg/100gm of pulp can be extracted from this fruit and is and can be used in several medicines. This fruit contents a high percentage of Vitamin C, tannin, medicinal properties and mineral contents of aonla offers enormous scope for processing. Chemical compositions of Amla/Aonla are given in Table I.

TABLE I: AMLA FRUIT CHEMICAL CONSTITUENTS [1]

Type	Chemical Constituents
Hydrolysable Tannins	Emblicanin A and B, Punigluconin, Pedunculagin, Chebulinic acid (Ellagitannin), Chebulagic acid (Benzopyaran tannin),Corilagin (Ellagitannin), Geraniin (Dehydroellagitannin), Ellagotannin
Alkaloids	Phyllantine, Phyllembein, Phyllantidine
Phenolic compounds	Gallic acid, Methyl gallate, Ellagic acid, Trigallayl glucose
Amino acids	Glutamic acid, Proline, Asparic Acid, Alanine, Cystine, Lysine
Carbohydrate	Pectin
Vitamins	Ascorbic acid
Flavonoids	Quercetin, Kaempferol
Organic acid	Citric Acid

WINE AND ITS ORIGIN

As per Archaeological evidence it reveals that at the earliest known, wine made by fermenting grapes, took place from the late Neolithic or early Chalcolithic, possibly at early sixth millennium BC, between the Caucasus and the Middle East, with evidence of wine production at different locations dated 6000 BC in Georgia, 5000 BC in Iran, and 4100 BC in Armenia. In an extensive gene-mapping project (2006), archaeologists analyzed the heritage of more than 110 modern grape cultivars, narrowing their origin to a locality in Georgia, where wine residuum were also detected on the inner surfaces of 8,000-year-old ceramic storage jars. Chemical analysis of 7,000-year-old broken pieces of pottery indicated early

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winemaking in the Neolithic village of Hajji Firuz Tepe in Iran's Zagros Mountains. Other noteworthy areas of wine production have been observed in Greece and date back to 4500 BC. The same sites also comprise the world's earliest evidence of crushed grapes. The primary known notice of grape-based wines in India is from the late fourth century writings of Chanakya, the chief minister of Emperor Chandragupta Maurya. In his writings, Chanakya censures the use of liquor while chronicling the emperor and his court's successive liberality of a style of wine known as *madhu*. In China, in the early years of the seventh millennium BC about 9,000 years ago, an archaeologist indicates a possibility that grapes were mixed with rice to produce mixed fermented beverages. [2]

Wine is a mixed drink produced using grapes and depending on the definition of "made from grapes" there are at least two independent inventions of the wine. A typical wine contains ethyl alcohol, acids, sugar, tannins, higher alcohols aldehydes, esters, anthocyanins, vitamins, amino acids, minerals, minor constituents like flavoring compounds etc. [4]. This is probably the most ancient fermented beverage and was found to be mentioned in the Bible and in other documents from Asian countries.

Fig 1:16th Century Wine press [2]

Fig 2: Pressing wine after the harvest [2]

CLASSIFICATION OF WINE

Depending upon the various factors such as cultivar stage of ripening of fruits, chemical composition of juice, use of additives to the must, vinification techniques the conversion of grape juice or other vegetable extract into wine by fermentation and ageing of wine, the alcohol and sugar content, the wines are classified as natural wines (9-14 % alcohol) and dessert and appetizer wines (15-21 % alcohol). Dry wine, sweet table wine, specialty wine, champagne, muscat and burgundy wines (natural wines) while vermouth, sweet wine, cherries and port wines are regarded as dessert and appetizer wines [3]. Red and white wines are most legendary, followed by rosé and sparkling wines. Around the world, there are additional wine specialties, such as the Portuguese Port Wine, a very rich flavor, often used by chefs in their signature dishes. Classification of wines are grape wine, fruit wine, berry wine, vegetable wine, plant wine, raisin wine. Grapes are used for the production of grape wine. Fruit wines are also made from other ingredients (pear, apple, banana, papaya, mango, jackfruit juice, etc) than grape. Cherries which have sufficient acidity to wine that can be used for wine production. Plants such as maple, birch, melons, watermelons and other garden plants such as rhubarb, parsnips and rose petals are those plants from which plant wine is produced. Dried grapes (raisins) are used for production of raisin wine. Combination of grapes and wine materials produced] Multisort wine. Different types of wine can be classified as red, white and pink wine on the basis of time of fermentation grape varieties and color fruit wines are in red. Fig 3 shows the Red wine and white wine. [4]

Fig 3: Different type of wines [4]
(a) Red Wine (b) White wine

A. Red wine

Generally, red wine is appeared in black color. It is produced by using red grapes. There are many different types of red wines. In the kingdom of wines, red wine is considered to be the most standard wine. Added ingredients like aromas, eucalypti, chocolate or even mint hints give the more taste to the red wine. The juice produced using black grapes is generally greenish-white in color; due to presence of anthocyan pigments present in the skin of the grape.

B. White Wine

Color of white wine is depends on the skin of the grape. This wine is generally in yellow, gold or straw colour. White wine can be produced by the alcoholic fermentation of the non-coloured pulp of green or gold coloured grapes or from selected juice of red grapes, which produced in Europe and numerous other places such as Australia, California, South Africa and New Zealand. White wines often taste lighter, crisper and more refreshing than a red.

FERMENTATION

Fermentation is the term to describe any process for the production of a product using culture of a microorganism. The first occurrence of fermentation process was observed by humans. Initially, Fermentation word was utilized as a part of connecting to winemaking was in reference to the clear "boiling" that came from the anaerobic reaction of the yeast to the sugars in the grape juice which released carbon dioxide. The Latin *fervere* means, literally, *to boil*. In the mid-19th

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century, the connection between yeast and the process of the fermentation in which the yeast act as catalyst and middle person through a progression of a reaction that convert sugar into alcohol was observed by Louis Pasteur. The discovery about the alcohol in the early 20th century contributed more to the perceptive of the complex chemical processes involved the conversion of sugar to alcohol.

The term 'wine' is applied here to the product made by the alcoholic (ethanolic) aging of sound grape juice by yeast (Saccharomyces cerevisiae), followed by an appropriate period of ageing. The fermentation reaction of hexose sugar (glucose) by yeast has been by Gay-Lussac which forms the basis of calculating fermentation efficiency.

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

Glucose	Ethanol	
(1 Kg)	(0.551 kg)	(0.489 kg)

BATCH FERMENTATION

Batch fermentation is generally carried in a closed system. In the fermentor, the sterilized nutrient solution is to be inoculated, at time t=0, with a given number of cells microorganisms and incubation is allowed to continue. In the entire process of fermentation, only oxygen is added in case of aerobic microorganisms, an antifoam agent. An acid or base is to be added to control the pH. As the process of fermentation proceeds, the composition of the biomass, the metabolite concentration and culture medium generally change constantly due to metabolism of the cells. [5]

During growth of microorganisms in batch culture, four typical phases of growth are observed after the inoculation under physiological conditions. The population growth of bacteria is monitored over a period of time, plotting data of cell numbers with time, will yield a typical bacterial growth curve.

SCENARIO: AVAILABILITY OF FRUITS CROPS IN MAHARASHTRA REGION

For the cultivation of a wide range of crops, agro climatic condition of Maharashtra is appropriate for the progressive farming. The State has a large urban population density. Maharashtra is the backbone for cultivation in India, with more than 13 lakh ha under various fruit crops. [6]. In Maharashtra, for the year 2008-09 gross State Domestic Product (GSDP) at current prices is estimated at Rs. 692479 crores and contributes more than 13% cent of national GDP. Agriculture and unified exercises contribute nearly 12% per cent to the State's income. Thus, in general, in the area of agricultural industries, Maharashtra is the mainstay for food processing in particular. There is a gigantic potential for much higher quality expansion through processing. The potential fruit crop production clusters is given in the Table II. As per agro industrial policy, the major fruit producing crops are Pomegranate, Banana Orange Grapes Mangoes Amla.

TABLE II: LIST OF POTENTIAL AGRI-PROCESSING CLUSTERS IN MAHARASHTRA [7]

Clusters	Crops	Locations
Fruits	Pomegranate	Solapur, Nashik, Sangli, Pune, Satara
	Banana	Jalgaon, Nanded, Hingoli, Nandurbar, Pune
	Orange	Amravati, Nagpur, Jalna, Yavatmal, Nanded
	Grapes	Nashik, Sangli, Ahmednagar, Solapur, Pune
	Mangoes	Sindhudurg, Ratnagiri, Raigad, Aurangabad, Thane
	Amla	Akola, Yavatmal, Jalna, Dhule, Buldhana

Source: FAO Website and for India: Horticulture Division, D/o Agriculture & Cooperation

As per National horticulture board, the highest production of horticulture crops is found to be 2462 MT in the year 2010-2011, 2510 MT in the year 2011-2012 and 3198 MT in the year 2012.

TABLE III: AREA AND PRODUCTION OF HORTICULTURE CROPS FOR THE YEARS (2010-11, 2011-12 AND 2012-13) [8]

Fruit Crops	2010-11		2011-2012		2012-2013	
	A	P	A	P	A	P
Amla	67	677	95	961	108	1266
Grapes	112	1235	116	2221	118	2483
Guava	205	2462	220	2510	236	3198
Jackfruit	36	540	60	1042	67	1176
Kiwi	0	1	3	6	4	7
Litchi	78	497	80	538	83	58
Plum	14	32	26	72	24	74
Pomegranate	107	743	112	772	113	745

(A: Area in '000 Ha; P: Production in '000 MT)

Table IV: PRODUCTION SHARE OF LEADING FRUIT PRODUCING STATES IN 2012-13 [9]

Sr. No	States	2012-2013	
		Production (in '000 MT)	%Share
1	Andrapradesh	13939.1	17.1
2	Maharashtra	9785.0	12.0
3	Gujarat	8413.2	10.4
4	Tamilnadu	6699.9	8.2
5	Karnataka	6619.6	8.1
6	MadyaPradesh	5450.0	6.7
7	Uttarpradesh	5176.1	6.4
8	Bihar	4249.1	5.2
9	West Bengal	3172.5	3.9
10	Kerala	2583.9	3.2
11	Others	15196.9	18.7
12	Total	81285.3	100.0

Source: Horticulture Division, D/o Agriculture & Cooperation

WORLD SCENARIO

TABLE V: MAJOR FRUIT PRODUCING COUNTRIES IN THE WORLD (2012-13) [10]

Country	Area in '000 Ha	Production in '000 MT	Productivity in MT/Ha
China	11834	137067	11.6
India	6982	81285	11.6
Brazil	2325	38369	16.5
United States of America	1138	26549	23.3
Indonesia	797	17744	22.3
Philippines	1240	16371	13.2
Mexico	1257	15918	12.7
Turkey	1103	14975	13.6
Spain	1539	13996	9.1
Italy	1126	13889	12.3
Others	27925	270592	9.7
World	57265	646758	11.3

Source: FAO Website and for India: Horticulture Division, D/o Agriculture & Cooperation

The term 'fermentation' is gotten from the Latin verb, *fevere*, to boil. Fermentation technology is one of the oldest food innovations that have been utilized for a few thousand years as an effective and low cost means for preserving foods and beverages [11]. Food fermentation is of prime importance in the developing countries where the limitation of resources empowers the utilization of locally accessible fermented food products for additional nutrition. These fermented products are more regular among individuals belonging to rural areas, without much awareness about the microflora involved in their production. In the previous couple of years, extraordinary accentuation has been given to identify unknown microflora associated with these products. This microflora includes a mix of microscopic organisms, yeast and fungi which have been reported by several specialists from different fermented foods viz. kinema (Kim-Bong-Joon 2000) [12], bushera [13] and togwa. [14]

The most vital microorganism connected with fermentation is yeast. Yeasts as a group of micro-organisms have been commercially abused as a fermentative species to complete alcoholic fermentation, especially *Saccharomyces cerevisiae*. The importance of this microorganism has urged to study the factors governing its growth, survival and biological activities in different ecosystems [15]. *S. cerevisiae* plays a prominent role in controlling the quality and flavor of the final product in wine fermentations [16] and thus it has received considerable attention in fermentation industry.

During literature survey, several papers have come into viewed which use different fruits such as apple, plum, jackfruit, kiwi, apricot, pomegranate, strawberry, kinnow, guava, jamun, sapota, litchi [17-28] for wine production. Pelczar *et al.* (1977) expressed that the species required in aging procedure is mostly *S. cerevisiae*. [29]. It is one of the most significant fungus in the history of wine production in the world. This yeast is responsible for the production of ethanol in alcoholic drink. The process produces ethyl liquor (ethanol) is the way of yeast to convert glucose into energy. *S. cerevisiae* has adjusted in a few essential ways and be able to break down their foods through both aerobic respiration and anaerobic fermentation. It can continue to exist in an oxygen deficient environment for a period of time.

II. MATERIALS AND METHODS

COLLECTION OF FRUIT AND EXTRACTS PREPARATION

Fresh amla fruits were gathered from the nearby market of Nagpur city, (M.S) India, during the month of October-December of year. They were washed in clean water and dried in air. The amla fruit was grated and the seeds were separated, the juice from the grated remainder was obtained by squeezing it in a muslin cloth. About 250 ml juice was obtained from 500 gm of amla. The juice was then stored in an air tight bottle to prevent invasion of any kind. Fresh juice was used for each experiment.

MICROORGANISM

The wine yeast Saccharomyces cerevisiae (stock culture of the Microbiological Labratory, RGBC Nagpur) was used in fermentation experiments. This yeast culture has previously been employed for making wine from different fruits and other substrates.[7-14]

INOCULUMS PREPARATION

A thermo tolerant, osmotolerant and alcohol tolerant strain of S. cerevisiae was used. The yeast was capable of growing up to a temperature of 45 °C in presence of 30 % sugar and 18 % ethanol. Baker's yeast was bought from the market in the form of spores a dissolved in warm distilled water to make it active. The cake yeast strains Saccharomyces cerevisiae was used in the study. Inoculum was prepared by adding a 5 gm of yeast to 100 ml of sterile MPYD liquid media taken in 250 ml flask under sterile conditions. The media was incubated at 26 °C for 24 h to prepare the starter culture. The cultures were maintained on malt extract, yeast extract, peptone, dextrose, and agar.

PREPARATION OF SAMPLES & WINEMAKING PROTOCOL

After fermentation, complete content of fermentor was dropped in a sterilized separating funnel. Bentonite (0.04 %) was added to remove the last remaining residues for clarification. Fermentor content mainly comprises water (boiling point 100 °C) and ethanol (boiling point 78.5 °C). By simple distillation ethanol (wine) is separated from rest of mass. Sodium metabisulphite was added as a preservative before bottling. The containers were loaded with wine, corked and sealed with beeswax. The procedure for making wine from amla fruit is shown in Fig.1.

FERMENTATION PROCESS

Post the shake flask study and distillation sample with the maximum yield was chosen for batch fermentation in 3 liter autoclavable fermenter and the sample volume was about 1liter.1:10 scale-up was done for fermentation. The various proportions used for the fermentation were as per the following table Amla fruits were washed in clean water and dried in air at atmospheric temperature for approximately one hour. The amla fruit was grated and the seeds were separated, the juice from the grated remainder was obtained by squeezing it in a sterilized muslin cloth. Approximately 500

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ml of juice was extracted from *1 kg of fruits.* Then tartaric acid and cane sugar were added to the juice (amelioration) to attain initially the total soluble solids (TSS) of 21 °Brix and pH of 4.54. The ameliorated juice was inoculated with one day old starter culture of Saccharomyces cerevisiae (2 %, v/v), and ammonium sulphate [$(NH_4)_2 SO_4$] at 0.1% concentration was added as an additional nitrogen source. Fermentation was carried out in a fermentor at ambient temperature (28 ± 2 °C) for seven days. Fresh juice was used for every run of the experiment.

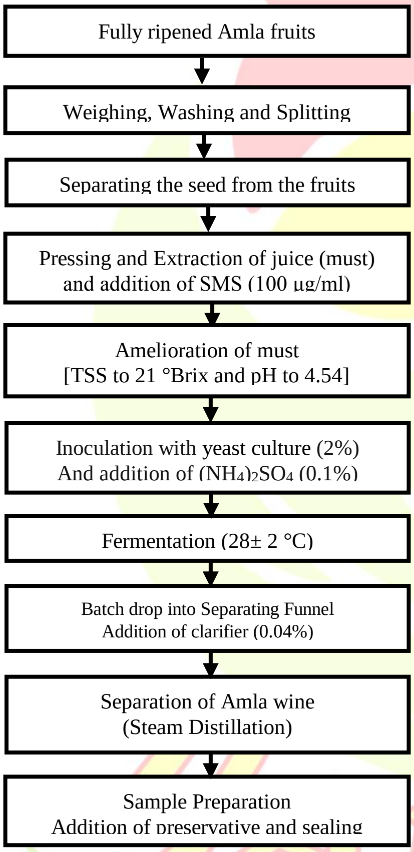

Fig. 4. Flow sheet of different unit operation in production of Amla wine

ANALYTICAL METHODS

$$\text{Fermentation efficiency was calculated as} = \frac{\text{Alcohol in wine}}{\text{Alcohol obtainable from sugar utilized}}$$

III. RESULTS AND DISCUSSION

EFFECT OF TEMPERATURE ON ETHANOL YIELD

During the ethanol production, temperature is one of the major factors. The fermentation process is an exothermic reaction which evolutes heat and raises the temperature of the fermenter. In industries, fermentation is typically carried out at surrounding temperature of 25 – 35 ºC but temperature exceeds 40 ºC during fermentation. Fermentation at above 40 ºC has advantages such as ethanol recovery and significant savings on operational costs of refrigeration control in distilleries for alcohol production. Therefore, most of the research studies have been carried out for development of yeast to ferment at high temperature of upto 40 - 45 ºC. To study variation of temperature for ethanol fermentation with respect to time, the samples were kept at different temperature 25 °C - 40 °C with addition of initial sugar concentration. The fermentation process was carried out in 250 ml flasks. During the fermentation process, ethanol sample were withdrawn like clockwork i.e. every 12 hours and the fermentation was done for 168 hours. A low ethanol yield of 6.5 % was observed at 25 °C in 168 hours. It was observed that the maximum ethanol yield is found to be at 30 °C as shown in Fig. 5. However, as temperature increasing beyond 30 °C, concentration of alcohol decreases. This may occur due to declining the growth of Saccharomyces cerevisiae. Hence, maximum ethanol yield was found to be at optimum temperature 30°C.

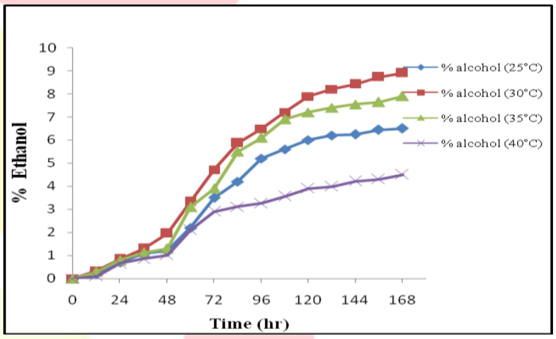

Fig. 5. Effect of temperature on Ethanol production

EFFECT OF PH ON ETHANOL PRODUCTION

Optimum initial sugar concentration and optimum temperature of 30 °C was selected for further studies and subjected to pH treatments 5 to 8. The results are shown in fig.6. At pH 5, fermentation took place but it gave low ethanol content. Best results were obtained at pH 5-6 where maximum ethanol production was noticed. The fermented samples were analyzed after each 12 hrs for pH measurement. As the fermentation process proceeds, the pH of sample decreased. It shows that as pH decreases, it inhibits the yeast multiplication. But sometimes, the resultant low pH ranging from 3.1-3.5 may help to keep the microbiological and chemical reactions properly controlled[18]. The samples were tested for fermentation with optimum sugar concentration and at ambient temperature. Yadav et al (1997) found an increase in alcohol concentration, productivity as well as effectiveness with a rise in pH from 4.0-5.0 and found that the optimum pH range for S. cerevisiae strain to be between pH 4.5 - 5.0. Based on fermentation efficiency the pH 6 was selected for further experimentation.

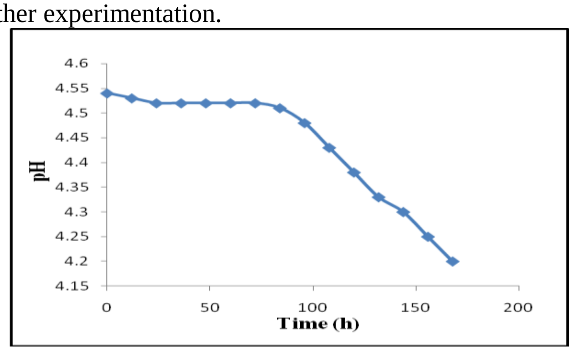

Fig. 6. Effect of pH on ethanol production

EFFECT OF SUGAR CONCENTRATION

Impact of sugar concentration on ethanol creation by S. cerevisiae has been studied and samples were withdrawn after every 12-hour interval and assessed for remaining sugars. Added yeast utilized the initial sugar concentration effectively for alcohol production and this experimental condition was maintained in fermentation broth for further study.

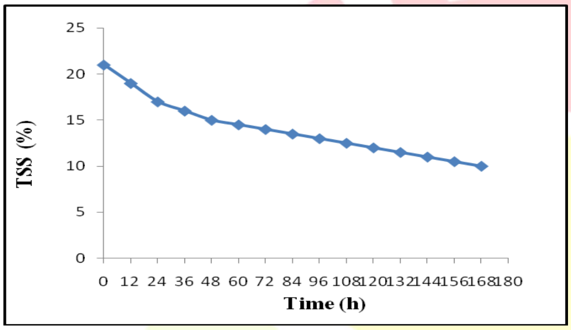

Fig. 7. Effect of sugar concentration on Ethanol yield

Optimum initial sugar concentration and optimum temperature of 30 °C was selected for further studies and subjected to pH treatments in the range of 5- 8. The results are shown in fig. 7. Fermentation carried out but it gave low ethanol content at pH 5. Best results were obtained at pH 5-6 where maximum ethanol production was noticed. The fermented samples were analyzed after each 12 hrs for pH measurement. As the fermentation process proceeds, the pH of sample decreased. It shows that as pH decreases, it inhibits the yeast multiplication. But sometimes, the resultant low *p*H ranging from 3.1-3.5 may help to keep the microbiological and chemical reactions properly controlled[18]. The samples were tested for fermentation with 20% sugar concentration and temperature of 29 ± 1°C. Yadav *et al* (1997) found an increase in alcohol concentration, productivity as well as effectiveness with an increase in pH from 4.0-5.0 and found that the optimum pH range for *S. cerevisiae* strain to be between pH 4.5-5.0. Based on fermentation efficiency the pH 6 was selected for further experimentation.

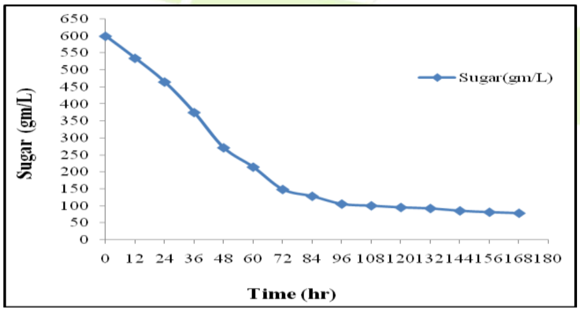

Fig. 8. Residual sugars present over the time

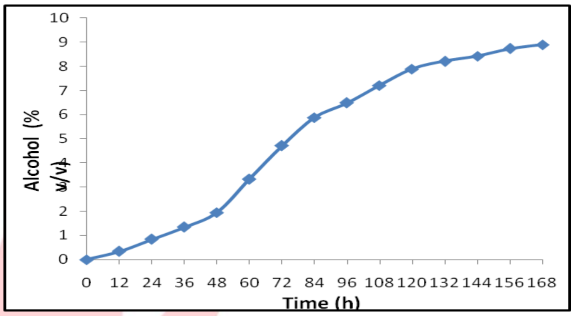

Fig. 9. Ethanol Production during Fermentation process

IV. CONCLUSION

Amla fruits can be used as a valuable ingredient for the production of an amla wine with all the important properties of wine having medicinal characteristics of amla fruits.

Characterization of Amla juice was done which shows that Amla is a rich source of vitamin C.

Temperature is one of the major constraints that determine the ethanol production. The fermentation process was carried out at 25, 30, 35 and 40 °C with 20 % initial sugar concentration. It was concluded that an increase in alcohol concentration, productivity as well as efficiency with a rise in pH from 4 - 5 and found that the optimum pH range for *S. cerevisiae* strain to be between pH 4.5-5.0. The fermentation process using *S. cerevisiae* under optimized conditions i.e. pH 6, optimum sugar concentration and temperature 30°C revealed an increase in ethanol production i.e. 8.9 % (v/v) with good fermentation efficiency.

However, as temperature increasing beyond 30 °C, concentration of alcohol decreased. This may occur due to declining the growth of Saccharomyces cerevisiae. Hence, maximum ethanol yield was found to be at optimum temperature 30 °C.

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