STANDARDIZATION OF POMEGRANATE WINE PRODUCTION BY USING COMMERCIAL STRAINS OF YEASTS

[1]CHARUSHILA V. DHAMANE, [2]VARSHA A. NIKAM, [3]KALYANI H. PAGAR, [4]SARITA U. MAHAJANI, [5]SHRADDHA S.KULKARNI

[1,2,3]Sinhgad College of Engineering, Department of Biotechnology, Pune
[4]Guide, [5]Co-Guide: (Assistant Professor)
E-mail: charudhamane@gmail.com, varshanikam@gmail.com, kalyanipgr@gmail.com

Abstract- Pomegranate fruit (punica granatum L.) is one of the most powerful, nutrient dense foods for overall good health. The characteristics of cultivars(such as, ganesha and bhagwa) of pomegranate has significant impact on the quality of wines .The effect of using mixed cultures of non-Saccharomyces and Saccharomyces cerevisiae yeasts in the physicochemical and Sensory qualities of the wines were analysed in this study. Based on growth curves, sugar consumption and glycerol production in pomegranate juice, Candida stellate 3433 was selected. This yeast was subsequently used in combination with S. cerevisiae var. ellipsoideus in pomegranate juice. A monoculture of S. cerevisiae was used as control. The wines fermented with mixed cultures had lower volatile acidity and ethanol concentration than the control. These characteristics positively influenced the sensory qualities of the wines produced with mixed cultures; the pomegranate juice fermentation was carried out by combination of free saccharomyces cerevisiae and immobilisation of candida stellate. It shows positive influence on final quality of wines

Index Terms- Cultivar, co-fermentation, pomegranate, wines, two different strains of yeasts.

I. INTRODUCTION

Pomegranates (Punica granatum L.) is one of the species in the Punicaceae family. There are different varieties of cultivars of pomegranates are available like Ganesh, Bhagwa, Ruby, Arakta and Mridula. In India, pomegranate is commercially cultivated in Maharashtra, Karnataka, Gujarat, Andhra Pradesh and Tamil Nadu. Pomegranate is an important source of potentially healthy bioactive compounds. The characteristics of cultivar depend on the growing region, climate, maturity, cultural practices and storage conditions. These cultivars have been selected based on consumer preference for high sugar to high acid ratios, and dark red rinds and arils. Pomegranate juice has become more popular because of the attribution of important biological actions (Lansky et al., 1998).

Thus, the antioxidant and antitumoral activity of pomegranate bark tannins (punicacortein) (Kashiwada et al., 1992; Su et al., 1988) and the antioxidant activity of the fermented pomegranate juice (Schubert et al., 1999) have been reported. In this study cultivar bhagwa and Ganesha has been used for the production of wine. Bhagwa has dark red arils which gives best appearance to final wine and Ganesha has good sugar content which is beneficial for fermentation process. In wine production, yeasts are responsible for the conversion of sugar into ethanol, carbon dioxide and hundreds of secondary products that collectively contribute to the quality of the product. Hence, these microorganisms may have a positive or negative influence in the sensory traits of the product. Although non-Saccharomyces yeasts were long considered harmful, evidence in recent years has shown that their use may give complex organoleptic characteristics to the wine, thus increasing its quality, because they produce compounds such as glycerol, isoamilic alcohol, succinic acid, acetic acid and propanol that affect the sensory characteristics of the product. Candida stellate produced higher glycerol concentration compared to the other microorganisms used, including Saccharomyces cerevisiae.

During recent years, a new concept of “restricted alcoholic fermentation” has come to attention. In this procedure, production of alcohol during the fermentation process is reduced from the very beginning. This may be achieved either by using yeast that can only partially ferment juice or by repressing or interrupting fermentation with different compositional and/or process factors such as the heat treatment. For low-alcohol production, strain of the yeasts, as well as the predominant fermentable sugar in the yeast growth medium is among the most important factors affecting the performance of alcohol production.

Thus, the present study is aimed at investigating the fermentation characteristics of combination of two strains such as non-saccharomyces candida stellate and saccharomyces strain like S cereviase in MYGP broth medium containing different sources (carbon, nitrogen, sugar, minerals, etc.) in order to consider the suitability of treatments (strains/sugars) for production of non-alcoholic wine as well as to devise treatments resulting in greatest growth rate of yeast cells. It would help to develop culture media multiplication, transformation and maintenance of the yeast starters in research and industrial laboratories.

II. MATERIALS AND METHODS

2.1. Micro-organism and media
S. cerevisiae var. ellipsoideus NCIM NO 3215 and C. stellate NCIM NO 3433 were supplied by National Chemical Laboratory, (pune) in freeze-dried and slant forms. The starters were propagated in MYGP broth .The cultures were kept in refrigerator (5°C) until used. Subcultures were made every 2 weeks in order for starter renewal using MYGP broth.

The pomegranate fruits of Ganesha, bhagwa cultivars were collected from market yard district of Maharashtra. The fruits after gentle washing in water were used for wine preparation in the laboratory scale flask fermenters.

2.2 Pomegranate juice samples
Pomegranates were cut in halves and juices of each cultivar were obtained by hand pressing and straining through double fold musLin cloth. Bhagwa and Ganesha freshly prepared juices were also mixed 1:1, to study the behavior of the blended juice.

2.3 Immobilization
c.stellata cells for immobilization were grown in MYGP at 250 c in a rotary shaker (150 rev min for 72 h, harvested by centrifugation, washed three times with sterile distilled water, and added to 2.5%sodium alginate at a ratio of 5%(wet weight) The beads were formed and kept for 1 hr and washed several times with sterile distilled water and used immediately.

2.4 Starter culture preparation
In case of wine preparation from pomegranate, adjustment of brix of the juice is generally required in order to have sizable amount of alcohol in wine. Hence in present study T.S.S. content of known volume of juice was adjusted to required Brix by addition of 5% of sucrose.

The acidity was adjusted to 0.9 per cent by addition of citric acid so that the pH of the must will be around 3.5 which is required for better fermentation and better quality of wine.

After adjusting T.S.S., acidity the juice was distributed in conical flasks each containing 30 ml juice and pasteurized between 82 and 850 C for 20 minutes. Each 30 ml pasteurized juice after cooling was inoculated with two loopful of pure culture of Saccharomyces cerevisiae var. ellipsoideus (NCIM – 3215) under aseptic conditions. The flasks were incubated at 28 ± 20 C for 24 hours. These 24 hours old starter cultures were used for the experiments on wine making by pouring 30 ml starter culture (5%) in 600 ml juice.

2.5 Winemaking procedure:
Duplicate fermentations were carried out of pomegranate juice of cultivars of Ganesha and bhagwa and blend (combination of bhagwa and Ganesha at the ratio of 1:1) under static condition at 25°C in mini fermenters. Yeast cultures of Saccharomyces ellisoideus and candida stellate were added separately. The 5 per cent yeast inoculum was added as starter culture and allowed to ferment at room temperature. Sequential fermentation, C. stellate immobilized cells (20% (w/v) of beads) with the addition of S. cerevisiae (1*106 cells ml -1) after 3 days was carried out. The cotton plug was replaced after 24 hours from lab. The temperature was kept at room temperature during the fermentation process .After completion of the fermentation in 2-3 weeks, racking was done 4 times at weekly intervals. After final racking, wines were clarified again by adding 400 mg/litre bentonite clay Then, the wines were transferred to new glass vessels, Samples were collected at days 0, 2, 3, 4, 5, 6 (during juice fermentation),7, 8, 9 (during the end of the fermentation), 10 (when racked and clarified), 13, 16 and 20 (during wine stabilization). Samples (7 mL) were taken from each vessel at sampling time and stored frozen (-20^{0}C) until analysis. The clear wine samples were bottled and tightly corked without leaving headspace and kept for maturation at 15-16˚C.

III. RESULTS AND DISCUSSION

Quality parameters of juices and wines Titratable acidity (TA) (expressed as g/L of citric acid), pH, and alcohol were determined as oenological quality parameters.

3.1. Quality parameters of juices
The results (Table 1) revealed that the bhagwa cultivar having maximum waste as compared to Ganesha. hence after crushing Ganesha yielded the maximum yielded juice of 450 ml per kg of fruits followed by Ganesha 350 ml. Each part of pomegranate fruit can be used for by- products formation .The seeds can also be used for the oil extraction.

TABLE 1 A) Physical Parameters of Juice

Sr No	Parameters	Pomegranate cultivars	
		Bhagwa	Ganesha
1	Fruit colour	yellow to red	Pink
2.	Arils colour	Dark red	Pink
3.	Average fruit weight (g)	350.27	216.5
4.	Aril waste	217.8	98.17
5.	Fruit waste (g/kg)		
	a Seed waste	39.60	17.66
	b Rind waste	149.8	70.9
6.	Percent seed /kg fruit	11.30	8.15
7.	Juice yield	450 ml	350 ml

The (table 2) revealed that the pH of fruit juices of all the two cultivars was found in the range of 3 to 3.02

and hence required to be raised to a pH of 3.5 by the addition of sodium chloride for better quality wine production.in pomegranate mostly malic and tartaric acids are present .The titrable acidity expressed in terms of tartaric acid. The titrable acidity was found maximum in Ganesha and blend (0.7 %).The sugar content in blend was found to be maximum as compared to bhagwa and ganesha.

TABLE 1 B) Chemical Parameters of Juice

		Pomegranate cultivars	
Sr No	**Parameters**	**Bhagwa**	**Ganesha**	**Blend**
1.	Titrable acidity	0.52	0.67	0.67
2	malic acid	0.46	0.59	0.59
3.	pH	3.22	3.12	3.16
4.	Total sugars (gm/l)	100.98	112.26	217.01

3.1. Quality parameters of wines

After extraction of juice, pilot fermentation was carried out in such ways that focus on chemical addition, dilution and pasteurisation effect to corroborate its effect on wine quality. For pilot fermentation, at the beginning we choose the bhagwa cultivar. The pilot fermentation was carried out to examine the strength of c stellata for fermentation of pomegranate wine.c.stellata was added in immobilised and free form. 4 sets were adjusted along with control. Control consist of undiluted, unpasteurized, immobilised c stellate. First set consisted of set pasteurized, diluted, immobilised c .stellata and addition of chemicals .Second set consisted of unpasteurized ,undiluted, immobilized c stellata and without addition of chemicals.

Methanol presence test (litmus paper) No colour change

chemicals .Second set consisted of unpasteurized ,undiluted, immobilized *c stellata* and without addition of chemicals.

Third set consisted of unpasteurized, undiluted, free *c.stellata* and addition of chemicals. Forth set consisted of consisted of pasteurized, diluted, and free *c.stellata* with addition of chemicals.

Pilot results

Parameters	**control**	**1**	**2**	**3**	**4**
1. Tirable acidity	0.9	0.8	0.9	0.7	0.9
2. sugar content	0.0	0.0	0.2	0.0	0.1
3. pH	3.2	3.24	3.48	3.05	3.13
4. Ethanol content	5	3	5.1	5.4	3.2
5. colour	dark red	orange	dark red	dark red	orange

According to these results, set three gives desired results and standard parameters such as undiluted, unpasteurized and with chemical addition. Has shown its positive influence on wine. After 10 days all sugar was utilised by the mixed culture comparatively then scale-up process carried out up to 600 ml. same procedure was implemented to Ganesha and blend .during scale up sequential fermentation is carried out .A culture of S. cerevisiae was used as control. In relation to the reduction of alcohol concentration in the mixed culture, there could be competition among the inoculated microorganisms which made the production of this metabolite less efficient. The wines were analysed for sugar reduction, pH, alcohol concentration, titrable acidity.The sugar content was determined using DNSA method. The potassium dichromate method was used to determine the ethanol content of wine.

Composition of base wine

Wine	alcohol (% v/v)	fermentable sugar (g/L)	pH	titrable acidity
Bhagwa	5.4	1.14		3.38
0.52				
Ganesha	6	0.38	3.34	0.9
Blend	5.1	0.38		3.32
0.52				

The analytical test are necessary to measure the quality of final wines which will be helpful for consumer acceptance. The table below shows

Test	**Observation**
Cold stability test	No settlings were seen
Heat stability test	No settlings were seen
Methanol presence test (potassium-di-chromate	Absence of acidic smell

CONCLUSION

Pomegranate (punica granatum L.) fruits are a very rich source of antioxidants and have numerous health benefits. This study provides information on the chemical composition of 'Bhagwa' and 'ganesha' pomegranate fruits which is an important commercial pomegranate cultivar. The search for new non-Saccharomyces isolates allows providing wines with distinctive qualities. The positive interaction of C. stellata immobilized cells and S. cerevisiae could be used profitably to produce specific wine. The trend in the wine industry is to develop new fermentation techniques and to produce alternative alcoholic beverages. In this context, the use of this biotechnological process could improve the quality of wine. we corroborated that the sequential fermentation gives better influence on quality of wine.

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