IJPSR (2017), Volume 8, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 16 March, 2017; received in revised form, 01 June, 2017; accepted, 25 June, 2017; published 01 November, 2017

OPTIMUM PARAMETERS FOR WINE PRODUCTION FROM POMEGRANATE FRUIT JUICE

Samson, Ashok Kumar Singh and Gauri Singh*

Department of Microbiology, Dolphin (PG) Institute of Biomedical & Natural Sciences, Dehradun-248007, Uttarakhand, India.

Keywords:

Alcohol, Fermentation, Residual Sugar, Pomegranate wine

Correspondence to Author:
Dr. Gauri Singh

Department of Microbiology Dolphin (P.G) Institute of Biomedical and Natural Science Manduwala, Dehradun - 248007, Uttarakhand, India.

E-mail: gaurisingh2007@gmail.com

ABSTRACT: Pomegranate fruits are a very rich source of antioxidants, Vitamin B5 (pantothenic acid), potassium, flavonoids and have numerous health benefits. It may help decrease the risk of having a heart disease, heart attacks, and strokes. These characteristics of pomegranate have great impact on the quality of wines. So there might be a scope for large scale manufacturing of the pomegranate wine as it has many health benefits than grape wine Thus, the present study is aimed at investigating the fermentation parameters of pomegranate wine. Fermentation of pomegranate was carried out by a yeast strain. The present study provides information on the parameters like inoculum size, pH, incubation temperature, substrate concentration and incubation period involved during wine production. These parameters are the part and parcel of wine production, without the knowledge of these parameters one cannot make a tasty and good quality wine. We see a significant variation between residual sugar % (w/v) & alcohol % by applying ANOVA for all parameters. Analysis reveals that best wine from pomegranate can be produced at an inoculum size of 8% v/v and pH 4.0 at the incubation temperature of 37 °C. Further, it can be defined better if we incubated neither less nor more than 7 days. Under these conditions, alcohol content of the wine reached to 10.1%. This study will provide a good reference for future industrial production of pomegranate fruit wine.

INTRODUCTION: Wine drinking has been a part of our lives for many centuries. Wine brings pleasure to the person who would drink it. It is incontestable that wine has an innate taste that can lift one´s spirit. Now a days, wine is not consumed only for pleasure. Many people make wine drinking a part of their routine because of its health benefits. Wine is packed with anti-oxidants that help fight different types of diseases and delay the signs of aging.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(11).4826-31

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (11).4826-31

Wine is an alcoholic drink made from fermented fruit juice. Generally, fruits contain quantities of sugar that can be used by yeast during the fermentation process. In addition to the inherent characteristics of fruit (pH values, sugar contents and nitrogen contents), other factors must be taken into account during fruit wine production. The initial sugar concentrations, fermentation temperatures, SO_2 concentrations and specific yeast strains are key factors in determining successful fermentative processes of fruit wine [1].

India is the second largest producing country of pomegranates. In the current study, pomegranate fruit was used to develop wine by fermentation. Pomegranate has long been regarded as a promising diet source of phytochemicals, including

ellagitannin, flavonoids, anthocyanins [2] which possess several health promoting characters, like maintaining redox balance of internal environment, prevention and treatment of cardiovascular disease, diabetes, cancers, Alzheimer`s [3]. It is an abundant source of various Vitamins. Pomegranate is a fine, natural source of Vitamins A, C and E. It also contains folic acid. Pomegranate wine as an emerging beverage not only offers an alternative to employ underused pomegranate fruits [4], but also provide multiple beneficial effects on health management [5].

The soluble polyphenolic content of pomegranate juice (0.2 to 1.0%) includes anthocyanins, catechins, tannins, and gallic and ellagic acids [6]. The pomegranate wine has up to 3 times more antioxidants than red wine! Pomegranate wine has greater protection capacity than red wine on low-density lipoprotein oxidation [7].

An efficient ethanol production requires four components: fermentable carbohydrates, an efficient yeast strain, a few nutrients and simple culture conditions. The yeast cell contains enzyme catalysts that provide an energetically favourable pathway for the reaction. At the moment, most of the wine production processes are relying on *Saccharomyces cerevisiae* strains that allow rapid and reliable fermentations, reduce the risk of sluggish or stuck fermentations and prevent microbial contaminations [8]. Yeast starter cultures that are specifically selected for the winemaking process on the basis of scientifically verified characteristics typically complement and optimize the raw material quality and individual characteristics of the wine, producing a more desirable product [9].

We defined factors namely inoculum size, temperature, pH, substrate concentration and incubation period to achieve the optimum fermentation condition for pomegranate wine.

MATERIALS AND METHODS:

Culturing of yeast: The yeast strain *Saccharomyces cerevisiae* (MTCC-36) was obtained from IMTECH Chandigarh and taken as model strain. The cultures were kept in refrigerator until used.

Preparation of inoculums: The yeast strain was inoculated in the glucose yeast extract broth .The inoculated broth was incubated at 37 °C in orbital shaker for 48hr. After 48hr the count was done using haemocytometer to check the optimum number of yeast cells. The broth with spore count 3 x 10^8 was taken.

Preparation of pomegranate juice: The fresh pomegranate fruits were collected from the fruit vendor shop Premnagar, Dehradun. The juicy arils were separated from fresh fruits with the help of stainless steel knife. The arils were crushed to juice in fruit mixer. 700 ml of juice was collected from 2 kg of pomegranate fruit.

The juices were transferred into five 250ml conical flask with each conical flask containing 100ml of juice. The inoculum was added to juice and allowed for fermentation.

Optimization of culture parameters:

Inoculum size: Keeping all the parameters constant the inoculums ((*Saccharomyces cerevisiae*) of different size *i.e.* 2%, 4%, 6%, 8% and 10% (v/v) were added into the juice and incubated for 7 days.

pH: The pomegranate juices were incubated with taking optimized inoculum size but at different pH *viz;* 3.0, 3.5, 4.0, 4.5, 5.0 for 7 days.

Temperature: The pomegranate juices were incubated with taking optimized inoculum size and pH but at different temperature *viz*; 25°C, 27°C, 30°C and 37°C for 7 days.

Substrate concentration: The pomegranate juices were incubated with taking optimized inoculum size, pH and temperature but with different substrate concentration *viz*; 20%, 40%, 60%, 80%, 100% (v/v). After 7 days the substrate concentration was optimized.

Incubation period: The pomegranate juices were incubated with taking optimized inoculum size, pH, temperature and substrate concentration but for different incubation period (5 days, 10 days and 15 days). After 15 days the incubation period was optimized. Glucose yeast extract broth was taken as control for optimization of above parameters.

Estimation of residual sugar content: The residual sugar content of the fermented juice was estimated by the DNSA method [10].

Estimation of Alcohol content: The Alcohol content of the fermented juice was estimated by Dichromate Titration method [11].

Statistical Analysis: Data analysis was done using ANOVA.

RESULTS AND DISCUSSION: The quality of wine can be characterized by its ethanol content which is the chief component found in all types of wine [12]. It is worthy to note that final sugar concentration has inverse relationship with ethanol concentration. Therefore, in the current study pomegranate juice were optimized for wine production for above factors. The different parameters were studied after 7 days of Incubation. We let a null hypothesis between residual sugar % & alcohol % for statistical analysis

Inoculum size: The optimization of inoculum size is important as sugar consumption is a balance between biomass development and ethanol production and a high inoculum size will thus be a compromise on amount of ethanol produced. The pomegranate juice were incubated with different inoculums size *viz*; 2, 4, 6, 8 and 10%. We observed that ethanol production increase with increase in inoculums size up to 8% and decreases beyond this level while reverse were found in reducing sugar concentration (**Table 1**). It was, therefore, concluded that 8% inoculum is the optimum and selected for further studies.

TABLE 1: OPTIMIZATION OF INOCULUM SIZE FOR ALCOHOL PRODUCTION AND RESIDUAL SUGAR

S. no	Inoculum(%)	Residual Sugar% (w/v)	Alcohol(%)
1	2	5.89	5.6
2	4	5.75	6.8
3	6	5.55	7.7
4	8	5.80	9.2
5	10	5.60	7.7

Summary

Groups	Count	Sum	Average	Variance
Column 1	5	28.59	5.718	0.01987
Column 2	5	37	7.4	1.755

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7.07281	1	7.07281	7.969947	0.022386	5.317655
Within Groups	7.09948	8	0.887435			
Total	14.17229	9				

When we take Inoculum % as a single factor and apply ANOVA to find out whether there is any variation, we see a significant variation between residual sugar % (w/v) & alcohol % (p-value=0.022386<0.05) and ($C_{alculated}$ =7.969947 > $C_{ritical}$=5.317655), which rejects the null hypothesis. The outcome of this study was almost similar to that of other studies v/v. Similar trends have also been reported by Singh and Kaur (2009) [13]. Where they observed 10% (v/v) as optimized inoculum level for litchi wine production. An optimized inoculum level of 10% v/v for alcoholic fermentation of jamun, plum, apple, pear juice, guava and 7.5% inoculum size for kinnow wine production has been observed in other research reports.[14]

Effect of pH: The pH of juice is important parameter for the successful progress of fermentation. Control of pH during wine fermentation is important for two reasons. The growth of bacteria is retarded by acidic solution and yeast grows well in acidic conditions [15]. So, different pH ranges from 3.0 to 5.0 was tested It has been observed in pomegranate wine, alcohol production significantly increase with increase in pH up to 4.0, and decrease beyond that pH level 4.0. (**Table 2**).

TABLE 2: OPTIMIZATION OF PH FOR MAXIMUM ALCOHOL PRODUCTION AND RESIDUAL SUGAR

S.no	pH	Residual sugar % (w/v)	Alcohol conc. (%)
1	3.0	2.65	6.6
2	3.5	2.85	7.1
3	4.0	2.18	9.8

4	4.5		2.50			7.3
5	5.0		3.80			6.4
Summary						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Column 1	5	13.98	2.796	0.37483		
Column 2	5	37.2	7.44	1.873		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	53.91684	1	53.91684	47.97235	0.000121	5.317655
Within Groups	8.99132	8	1.123915			
Total	62.90816	9				

There is a significant variation between residual sugar % (w/v) & alcohol % (p-value= 0.000121 <0.05) and ($C_{alculated}$ =47.972357 > $C_{ritical}$= 5.317 655) in case of pH. With increase in pH the ethanol content was reduced gradually, because yeast produce acid rather than alcohol with increase in pH [16]. The maximum alcohol production was found to be at pH 4.0 with alcohol content 9.8% v/v. However prior study on screening of pomegranate for wine production. Matapathi *et al.*, 2004 [17]. was found to be at pH 2.9-3.4 with maximum alcohol content 12.9% v/v.

Effect of Temperature: Temperature can affect the sensitivity of yeasts to alcohol concentration, growth rate, rate of fermentation, viability, length of lag phase, enzyme and membrane function, etc. It has been observed that alcohol production was high at higher temperature.(**Table 3**) When we take Temperature as a single factor and apply ANOVA to find out whether there is any variation, there is a significant variation between residual sugar % (w/v) & alcohol % (p-value=0.334254>0.05) and ($C_{alculated}$ = 1.102027 < $C_{ritical}$=5.987378),

TABLE 3: OPTIMIZATION OF TEMPERATURE FOR MAXIMUM ALCOHOL PRODUCTION AND RESIDUAL SUGAR

S. no	**Temperature(°C)**	**Residual Sugar % (w/v)**	**Alcohol conc.(%)**
1	25	6.91	5.0
2	27	6.40	6.9
3	30	6.24	6.9
4	37	4.23	9.9

ANOVA: Single Factor Summary						
Groups	Count	Sum	Average	Variance		
Column 1	4	23.78	5.945	1.388833		
Column 2	4	28.7	7.175	4.1025		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3.0258	1	3.0258	1.102027	0.334254	5.987378
Within Groups	16.474	6	2.745667			
Total	19.4998	7				

As the temperature increases initial fermentation rate are increased due to the enzyme activity of the metabolic pathway. The current study showed the same optimum incubation temperature (37 °C) for maximum wine production as made by Torija, *et al*., 2001[18].

Effect of substrate concentration: Optimum substrate concentration is important for maximum alcohol production during fermentation. Substrate concentration is always related to enzyme activity of yeast which ferments the sugar content of substrate to alcohol. The pomegranate juice was adjust to different substrate concentration *i.e.* 20%, 40%, 60%, 80% and 100%. It was observed highest production of alcohol takes place in a 100% substrate (**Table 4**). Jadhav and Darikpar (2010) [19] found that maximum ethanol was produced at 40% concentration *Ficus glomerata* fruit.

When we take substrate concentration as a single factor, we see a significant variation between residual sugar % (w/v) & alcohol % (p-value = 0.21245 > 0.05) and ($C_{alculated}$= 1.835902 < $C_{ritical}$ = 5.317655.

Effect of Incubation Period: Incubation period is very important in wine production as it determines the ability of yeast sample to degrade the sugar content in the juice. The pomegranate juice was incubated for different periods of time *viz*: 3, 5, 7, 10, and 14 days respectively (**Table 5**).

TABLE 4: DIFFERENT SUBSTRATE CONCENTRATION AND THEIR RESPECTIVE SUGAR AND ALCOHOL CONTENT

S. no.	Substrate conc.(%)	Residual Sugar% (w/v)	Alcohol conc.(%)
1	20	3.00	1.7
2	40	3.40	3.3
3	60	3.40	5.6
4	80	3.60	6.1
5	100	3.70	10.1

Summary

Groups	Count	Sum	Average	Variance		
Column 1	5	17.1	3.42	0.072		
Column 2	5	26.8	5.36	10.178		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	9.409	1	9.409	1.835902	0.21245	5.317655
Within Groups	41	8	5.125			
Total	50.409	9				

TABLE 5: DIFFERENT INCUBATION PERIOD AND THEIR RESPECTIVE SUGAR AND ALCOHOL CONTENT

S. no	Incubation period(days)	Residual sugar% (w/v)	Alcohol conc.(%)
1	3	3.00	6.6
2	5	3.40	7.3
3	7	3.40	9.8
4	10	3.60	7.1
5	14	3.70	6.4

Groups	Count	Sum	Average	Variance		
Column 1	5	17.1	3.42	0.072		
Column 2	5	37.2	7.44	1.873		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	40.401	1	40.401	41.54344	0.000199	5.317655
Within Groups	7.78	8	0.9725			
Total	48.181	9				

There is a significant variation between residual sugar % (w/v) & alcohol % (p-value = 0.000199 <0.05) and ($C_{alculated}$=41.54344 > $C_{ritical}$=5.317655) with respect to incubation period. The incubation period for the maximum alcohol production was found to be 7 days in current study, however [20, 21] revealed that optimum incubation period was 10-14 days.

Similar work has been done by other workers by using different substrates such as Pooja and Kocher (2017) [22] Optimized the parameters on grape juice of Punjab MACS purple and H-144 cultivars that lead to 12.0 and 11.2 (%v/v) ethanol production, respectively. The results found in the work of Chakraborty *et al.*, (2017) [23] showed that the optimum conditions for production of wine from household wastes of vegetable peels having maximum yield 6.69% ethanol are temperature 32°C, pH 5.5 with incubation time of 2 days.

CONCLUSION: The results showed that the optimal condition for pomegranate fermentation was defined as inoculum size 8%, pH 4.0, temperature 37 °C, incubation period 7 days and 100% substrate concentration. Under these conditions, ethanol from the juice could be achieved reaching up to 10.1% (v/v), This study will provide a good reference for future industrial production of pomegranate fruit wine.

ACKNOWLEDGEMENT: The authors would like to thank to Mr. Vijay Ratan Pawar (Department of Biostatsitics Dolphin (PG) Institute

of Biomedical & Natural Sciences, Dehradun, India) for statistical analysis.

CONFLICT OF INTEREST: We declare that we have no conflict of interest.

REFERENCES:

1. Amerine M.A and Kunkee RE: Microbiology of winemaking. Annual Review of Microbiolgy 1968; 22: 323–358.
2. Fischer UA, Carle R and Kammerer DR: Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MS n. Food chemistry 2011; 127 (2): 807-821.
3. Landete J M: Ellagitannins, ellagic acid and their derived metabolites: a review about source, metabolism, functions and health. Food research international 2011; 44 (5), 1150-1160.
4. Pedro M, Amadeo GV, Nuria M and Cristina G: Pomegranate varietal wines: Phytochemical composition and quality parameters. Food Chemistry 2012; 133 (1), 108-115.
5. Fazeli MR, Bahmani S, Jamalifar H, and Samadi, N: Effect of probiotication on antioxidant and antibacterial activities of pomegranate juices from sour and sweet cultivars. Natural product research 2011; 25 (3): 288-297.
6. Adsule RN, Kotec PM and Kadam SS: Preparation of wine from pomegranate. Beverage and Food World 1992; 18:13-14.
7. Bakoyianis V, Kanellaki M, Kalliafas A and Koutinas A A: Low temperature wine making by immobilized cells on mineral kissiris. Journal of Agriculture and Food Chemistry1992; 40:1293-1296.
8. Romano P, Fiore R, Paraggio M, Caruso M and Capece A: Function of yeast species and strains in wine flavor. International Journal of Food Microbiology 2003; 86: 169-180.
9. Swiegers JH, Bartowksy EJ, Henschke PA and Pretorius IS: Yeast and bacterial modulation of wine aroma and flavour. Australian Journal of Grape Wine Research2005; 11:127-138.
10. Miller G L: Use of dinitrosalicylic acid for determination of reducing sugars. Anals of Chemistry 1959; 31: 426-428.
11. Williams MB and Darwin R: Colorimetric determination of ethyl alcohol Anals of Chemistry1950; 22:1556.
12. Joshi VK: Fruit wines. 2nd Ed. Directorate of extension, Dr. YS Parmer University of Horticulture & Forestry, Nauni, Solan, India,1998
13. Singh R S and Kaur P: Evaluation of litchi juice concentrate for Surface Methodology in optimization of process parameters for production of wine. Natural Product Radiance 2009; 8: 386-391.
14. Panesar PS, Panesar R and Singh B: Application of Response Surface Methodology in optimization of process parameters for production of Kinnow wine. Natural Product Radiance 2009; 8: 366-373.
15. Mathewson WS: The Manual for the home and farm production of alcohol fuel. In: farm production of alcohol fuel. J.A. Diaz publication. 1980.
16. Hodge M H and Hildebrandt M F: Alcoholic fermentation of molasses. In: Underkofler A.L, Hickey J.R (eds) Industrial fermentation. Chemical publishing Co. Newyork. 1954.
17. Matapathi S S, Patil AB, Nirmalnath P and Savalgi VV: Studies on Screening of Pomegranate Cultivars for Wine Production. Karnataka Journal of Agricultural Sciences 2004; 17(4):725-730.
18. Torija MJ, BeltranG, NovoM, Poblet M, Manuel J, Mas GA and Roze N: Effects of fermentation temperature and Saccharomyces species on the cell fatty acid composition and presence of volatile compounds in wine. International Journal of Food and Microbiology 2003; 85: 127-136.
19. Jadhav BL and Darikpar NV: Ethanol production from wild fruits of *Ficus glomerata*. bionao frontier 2010; 3(2): 167-171.
20. Borzani W, Gerab A, Higuera G A, Pires M H and Piplovic R: Batch ethanol fermentation of molasses: a correlation between the time necessary to complete the fermentation and the initial concentrations of sugar and yeast cells. World Journal of Microbiology and Biotechnology 1993; 9: 265-268.
21. Pooja. Optimization of fermentation conditions for production of wine from guava (*Psidium guajava* L.). M.Sc. Thesis. Punjab Agricultural University, Ludhiana, India. 2011
22. Nikhanj P and Kocher G S: Statistical optimization of ethanol fermentation parameters for processing local grape cultivars to wines. Journal of food processing and prervation 2017; 00: e13319 https://doi.org/ 10.1111/ jfpp. 13319
23. Chakraborty K, Raychaudhuri U and Chakraborty R: Optimization of bioprocess parameters for wine from household vegetable waste production by employing response surface methodology. International Food Research Journal 2017; 24(1): 48-55.

How to cite this article:
Samson, Singh AK and Singh G: Optimum parameters for wine production from pomegranate fruit juice. Int J Pharm Sci Res 2017; 8(11): 4826-31.doi: 10.13040/IJPSR.0975-8232.8(11).4826-31.