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Effect of pH on Physicochemical Parameters of Wine Produced from Banana

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A B S T R A C T

Wine is a fermentation product of mainly grape fruit juice or other fruit juices. Banana fruit juice can also be used for production of wine. Several factors affect alcoholic fermentation of must. Fermentation pH may also affect the quality of the wine produced from a given banana variety. The aim of the present study was to study the effect of pH on different physicochemical parameters of wine made from banana variety (grand naine). Banana must was prepared from banana fruit by treating juice with pectinase enzyme, diluting with water, ameliorating to 20 ^{o}Brix using sucrose and adding potassium metabisulfite. Then the pH of the must was adjusted as required. It was then subjected to pasteurization. The must was inoculated with yeast inoculum at 2 % concentration. Soluble solid and pH profile of the wine was monitored daily. After fermentation physicochemical parameters of the wine were analysed. It was found that the must fermented at lower pH gives highest percentage of alcohol.

Introduction

Wine is a fermentation product of mainly grape fruit juice or other fruit juices. pH plays an important role in wine fermentations. During fermentation of fruit juice into wine, yeasts utilise sugars of juice as substrates for their growth and convert them in to ethanol, carbon dioxide, and other metabolic end products. These constituents contribute to the chemical composition and sensory quality of the wine. Several factors affect alcoholic fermentation, such as addition of sulphur dioxide, temperature of fermentation, pH of the must, composition of fruit juice, inoculation with specific yeasts, and interactions of microorganisms (Torija, *et al.*, 2002, Jacobson, 2006).

The preferred wine pH is around 3.6 and the better pH for yeast and lactic acid bacteria is around 4.5. However spoilage bacteria can also grow well at pH 4.5. But, spoilage bacteria do not grow well below pH 3.6.Wine yeasts and some lactic acid bacteria can still metabolize in a pH range of 3.3–3.6. The low pH can prolong the fermentation process due to slow growth of microorganisms involved (Jacobson, 2006).

Fruit wine including grape wine is generally produced by alcoholic fermentation of respective fruit by different strains of yeast, *Saccharomyces cerevisiae*. Fruits such as pomegranates, mangoes, papayas, bananas, pineapples, guavas, kiwis, dates, and passion fruits are being utilized for production of wine (Reddy et al, 2012). In recent years, literatures on production and characterization of wine from banana fruit are also increasing (Akubor, et al. 2003, Isitua, et al. 2011, Byaruagaba-Bazirake, et al. 2013, Alvarenga, 2013, Cheirsilp and Umsakul, 2008). However, there is limited information available about the effect of pH on fermentation profile and various physicochemical parameters of banana wine.

Fermentation pH may affect the quality of the wine produced from a given banana variety. Thus it is necessary to investigate the effect of pH on fermentation of banana must. In view of this, here we have made an effort to study the effect of pH on different physicochemical parameters of wine made from banana variety (grand naine) available in local area of Nanded district of Maharashtra, India.

Preparation of Banana Juice

Bananas were procured from local market of Nanded, Maharashtra. Healthy and fully ripe banana fruits with black stem were chose for the study. For preparation of juice, fruits were washed with tap water, disinfected with 70% ethanol and peeled manually by hands. Pulp was weighed and cut in to thin slices. Paste of banana pulp was made and mix with boiled hot water in 1:3 proportion (pulp:water) in grinder-mixer.

The diluted pulp thus obtained was then treated with pectinase enzyme at a total concentration of 0.05% (w/v) of total juice volume for half an hour. Pectinase treated juice was kept in refrigerator (at 4°C) for about18 hrs for clarification. After clarification, the juice was filtered through double folded muslin cloth and again diluted by adding one volume of water in three volume of juice (water:juice, 1:3). The juice was ameliorated to 20 ^{o}Brix using sucrose and 5 mg/L of potassium metabisulfite was added to it. It is then distributed in to 350 ml aliquots in 500 ml flasks for pH adjustments. Must with following pH ranges were prepared to study the effect of pH on fermentation parameters: 3.0, 3.5, 4.0 and 4.5 in duplicates. The pH was adjusted with citric acid and calcium carbonate. The flasks containing juice were plugged with cotton and pasteurised in autoclave at 85-90^{o} C for 25 min. A control (pH 3.0) without pasteurization was also prepared. Must was then kept in refrigerator (at 4^{o} C) until required.

Preparation of Inoculum/ Starter Culture

Inoculum of the Baker's yeast (*S. cerevisiae*) was prepared as follows: seven gram Baker's yeast was inoculated into 350 ml of banana must and grown at room temperature (27-30 °C) for 68 hours. The cell number was counted by using haemocytometer. A cell density of approximately 3.2×10^{9} cells / mL was reached.

Fermentation

Must in each 500 ml flask was inoculated with 7 ml (2%, v/v) of 68 h old starter culture of *S. cerevisiae*. Fermentation was carried out at room temperature (25-28^{0}C) for 14 days. To monitor the progress of fermentation process and to observe the effect of pH on fermentation profile of must, soluble solid and pH of each fermenting must were measured on alternate days after 24 h of inoculation. Fermentation was stopped when there were no evolution of gas bubbles by keeping the flasks in refrigerator at temperature of 3-5^{0}C. Two replicates were maintained for performing the experiment.

Analysis

During the fermentation, the fall in TSS (°B), and the pH, were monitored at the appropriate time intervals. The wines were analysed for different physico-chemical characteristics. The pH of the must and wine was measured with a digital pH meter (Systronics, India), pre-calibrated with buffers of pH 4.0 and 7.0.

Titratable acidity was determined by titrating with 0.1 N NaOH and alcohol % by specific gravity method as described by AOAC. Total soluble solids (TSS) were determined using Abbey's refractometer (0–32) in terms of °Brix (Jacobson, 2006). Free SO_2 was determined by ripper titrametric method using iodine (Zoecklein, et al. 1995), and reducing sugars were determined calorimetrically using 3, 5-dinitrosalicylic acid (DNS) method as described by Nigam (2007).

Results and Discussion

We have investigated the effect of pH on the fermentation profile and physicochemical properties of banana must. The soluble solid and pH profile of banana must fermentation at different pH are presented in table 1 and 2 respectively.

Soluble Solids (^{o}Brix) Profile at Different pH

Soluble solid (^{o}Brix) represents the percent sugar and other dissolved solids in the solution. Decrease in soluble solids was observed during the fermentation of banana must. The rate of decrease was high in control without pasteurization, and in must with initial pH 4.0 and 4.5. The decreased was lowest and moderate in must with pH 3.0 and 3.5 respectively. The must fermented at all pH had reached up to 6 ^{o}Brix except the control which had slightly lowered 5.9 ^{o}Brix.

The results suggested that pH did not significantly affected the ^{o}Brix in fermented must and ultimately to the fermentation. However, pH could decrease the initial fermentation rate of banana must. Finally control without pasteurization could also had its microbial flora and might be responsible for utilization of dissolved solid i.e. sucrose.

pH Profile of Must

pH is important factor for the fermentation of fruit juice into a good quality wine. Low pH inhibits the growth of unwanted microflora and thus can improve the quality of final product. Complex pattern of pH variation was observed during fermentation of banana must at different pH.

During initial days of fermentation, pH of the must was increased in all treatment except in must with initial pH 4.5 which showed continuous fall and on last day a slight rise in its value. Banana must with pH 4.5 showed highest fall in pH on third day of fermentation. Significant fall was also observed in must with pH 4.0. The must showed alternate slight rise and fall in pH during fermentation. Pattern of rise and fall in pH was similar in both must with initial pH 3.0 and 3.5. However, final pH of the must with initial pH 3.0 was slightly increased from its initial value. Control without pasteurization also showed increased in pH from its initial 3.0 to final 3.3.

Table.1 Soluble Solid Profile of Must during Fermentation at Different pH

Soluble Solid (^{o}Brix) at different pH					
Day	**pH 3**	**pH 3.5**	**pH 4.0**	**pH 4.5**	**Control**
0	20.0 ± 0.0	20.0 ± 0.0	20.00 ± 0.0	20.00 ± 0.0	20.0 ± 0.0
1	19.80 ± 0.2	19.5 ± 0.71	19.00 ± 0.0	19.00 ± 0.0	19 ± 0.0
3	18.00 ± 0.01	16.75 ± 0.35	16.50 ± 0.0	15.85 ± 0.21	16.5 ± 0.3
5	13.5 ± 0.0	13.0 ± 0.0	13.25 ± 0.0	13.00 ± 0.0	13.0 ± 0.0
7	10.75 ± 0.30	11.0 ± 0.0	10.00 ± 0.0	10.10 ± 0.14	10.0 ± 0.0
9	8.8 ± 0.0	8.8 ± 0.0	8.80 ± 0.0	8.10 ± 0.14	8.0 ± 0.0
11	7.0 ± 0.0	6.5 ± 0.0	7.65 ± 0.91	6.50 ± 0.0	6.5 ± 0.5
13	6.0 ± 0.0	6.0 ± 0.0	6.00 ± 0.00	6.00 ± 0.0	5.9 ± 0.2

(Mean ± SD**):** Values are average of duplicates ± Standard deviation

Table.2 Ph Profile of Banana must during Fermentation at Different pH

Day	**3**	**3.5**	**4.0**	**4.5**	**Control**
0	3.01 ± 0.01	3.50 ± 0.0 ^{c}	4.00 ± 0.00^c	4.50 ± 0.0^c	3.00 ± 0.02
1	3.22 ± 0.02	3.69 ± 0.02	4.06 ± 0.01	4.47 ± 0.0	3.13 ± 0.01
3	2.84 ± 0.02	3.13 ± 0.00	3.21 ± 0.01	3.57 ± 0.04	3.18 ± 0.03
5	3.01 ± 0.03	3.21 ± 0.01	3.31 ± 0.01	3.56 ± 0.05	3.29 ± 0.03
7	3.00 ± 0.02	3.19 ± 0.01	3.25 ± 0.00	3.48 ± 0.04	3.26 ± 0.05
9	2.98 ± 0.01	3.16 ± 0.00	3.26 ± 0.02	3.45 ± 0.04	3.22 ± 0.02
11	2.99 ± 0.02	3.15 ± 0.01	3.21 ± 0.00	3.41 ± 0.01	3.18 ± 0.03
13	3.09 ± 0.02	3.27 ± 0.01	3.33 ± 0.01	3.53 ± 0.03	3.30 ± 0.01

(Mean ± SD**):** Values are average of duplicates ± Standard deviation

Table.3 Physicochemical Properties of Banana must after Fermentation at Different pH

pH	**Reducing Sugar (mg/ml)**	**% Tartaric acid**	**Alcohol %**	**Free SO_2 (mg/L)**	**Specific gravity**	**Soluble Solids (^{o}Brix)**
Must	68.50 ± 0.07	0.08 ± 0.03	ND	5.12 ± 1.42	1.082 ± 0.003	20.0 ± 0.0
3	2.37 ± 0.03	0.68 ± 0.01	7.32 ± 0.63	8.45 ± 0.0	0.990 ± 0.004	6.0 ± 0.5
3.5	2.42 ± 0.02	0.54 ± 0.04	6.67 ± 0.43	5.12 ± 0.0	0.990 ± 0.001	6.0 ± 0.0
4	2.09 ± 0.03	0.42 ± 0.0	6.46 ± 1.2	6.78 ± 1.27	0.989 ± 0.000	6.0 ± 0.0
4.5	1.96 ± 0.01	0.45 ± 0.04	6.71 ± 0.3	4.86 ± 0.36	0.990 ± 0.001	6.0 ± 0.6
Control	1.44 ± 0.05	0.53 ± 0.02	6.55 ± 0.52	5.12 ± 0. 91	0.989 ± 0.000	5.9 ± 0.5

Must without pasteurization
ND: Not Detected

Physicochemical Properties of Banana must after Fermentation at Different pH

Physicochemical properties are presented in table. Reducing sugar content of the must with initial pH 3.5 and 4.5 were found to be highest (2.42 mg/ml) and lowest (1.96 mg/ml) respectively. Control without pasteurization also contained very low amount of reducing sugar (1.44 mg/ml). Thus with increased in pH the utilization of reducing sugar by wine microflora also increased, except at pH range 3.0 to 3.5 where almost similar amount of reducing sugar was remained. Titratable acidity (% tartaric acid) of the must with initial pH 3.0 and 4.0 were found to be respectively highest (0.68 %) and lowest (0.42 %) after fermentation. Original must had very low titratable acidity (0.075 %). Fermentation of must resulted in to increase in titratable acidity. With increased in pH, titratable acidity of the fermented must was found to be decreased. Highest and lowest concentration of reducing sugars was observed in must fermented at pH 3.5 and 4.5 respectively.

Alcohol percent of the must with initial pH 3 was found to be highest (7.32%). At other pH alcohol percent was found to be lower.

With decrease in pH, alcohol percent does not increase linearly. However, low pH favours high alcohol production. This might be due to the inhibition of growth of other microbial flora at low pH and more alcohol production by yeast. Specific gravity of the must fermented at all pH almost had same value around 0.990. However, it is significantly lowered than the specific gravity of must. Concentration of SO_2 was also found to be almost similar in all must fermented at different pH. Soluble solid content was decrease in all fermented must at all pH.

Various factors such pH, temperature, concentration of sugars, etc can affect the physicochemical parameters of wine during its fermentation. pH is one of the important factor which affect the growth and metabolism of yeast. Generally acidic pH is favourable for wine microorganisms and the optimum pH for the growth of yeast and lactic acid bacteria is around pH 4.5. (Jacobson, 2006). Fall in pH and increase in titratable acidity was observed after fermentation of banana must. Similar observations were reported by other authors for banana wine (Onwuka and Awam, 2001, Akubor, et al, 2003). Metabolic activity of yeast and other microorganisms present in must probably be the responsible factor for the increase in titratable acidity and decrease in pH (Akubor, et al. 2003; Okoro, 2007; Alobo and Offonry, 2009; Panda et al. 2014; and Chowdhury and Ray, 2007). Banana must having pH 3.0 showed slight increase in pH value.

At all pH, decrease in soluble solid (^{o}Brix) was also observed. This decrease was due to utilization of sucrose by must microorganism. Similar results were found by Onwuka and Awam (2001) and Akubor, et al, (2003) for fermentation of banana must. However they reported very low soluble solid (^{o}Brix) as compared to our results.

The specific gravity of must was decreased after fermentation at all pH. This decrease was attributed to decrease in soluble solid (°Brix) and increase in alcohol % during fermentation by yeast. Free SO_2 in all fermented must remained almost constant. Optimum pH for production of alcohol during fermentation of banana must was found to be 3.0. In contrast to our results some authors have reported higher (Ranjitha, *et al.*, 2015, Onwuka and Awam, 2001) and lower alcohol percentage at high pH (Akubor, *et al.,* 2003) than we found. This might be due to the differences in experimental condition.

In conclusion, Study was carried out to determine the effect of pH on physicochemical parameters of banana must after fermentation. Must fermented at lower pH gives highest percentage of alcohol and titratable acidity. Further studies are needed to investigate the effect of other parameters on fermentation of banana must by using *Saccharomyces cerevisiae*.

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