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RESEARCH ARTICLE

BIOCHEMICAL AND MICROBIOLOGICAL CHANGES IN BANANA WINE DURING STORAGE

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Abstract

Wine was prepared from four different banana varieties (Khozikodu, Karpurchakra keli, Palaykondan, Alpan). Banana wine was stored in plain and ambercoloured bottles under ambient conditions for six months. After two months of storage, pH and alcohol content showed an increasing trend whereas titratable acidity, total soluble solids, and microbial population showed a decreasing trend. Same trend continue d even after two months of storage.After six months of storage period , the total soluble solids and alcohol content remained stable without showing any significant variation in their values as compared to the previous month whereas pH showed an increasing trend. Titratable acidity and microbial population showed a decreasing trend.Colour re tention was better in storage bottles that were amber coloured. Wine from the variety Alpan had better overall acceptability score compare d to other varieties throughout the storage period

Introduction: -

Banana (Musa Spp.) is one of the most important commodities in terms of volume of trade. Banana is cultivated in more than 150 countries across world. It is known by different names in various countries. Bananas are rich in carbohydrates and fibre. They contain various vitamins as A_1, B_1, B_2 and vitamin C. They are also rich in minerals such as potassium and possess good amount of starch and hemicelluloses. Compared to production of banana, post-harvest processing alternatives for it are less and losses are more. Post harvest losses of banana can be reduced by adopting proper post-harvest management practices and processing into value added products. Fermenting banana juice into beverages such as alcohol, beer, vinegar, and wine is considered to be an attractive means of utilizing surplus banana. Hence, the present study is aimed at evaluating wine produced by the popular banana varieties during storage for its microbial and biochemical parameters.

Materials and Methods: -

The present investigationwascarried out in the Department of Microbiology, NES, Science college, Nanded, Maharashtra, India using different varieties of banana. The different varieties used for the current study were Khozikodu, Karpurchakra keli, Palaykondan and Alpan. The fruit varieties were collected from Banana Research Centre, Nanded and kept for ripening under suitable conditions. Ripe fruits of banana varieties after removal of peel,

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were sliced into smaller pieces and added with boiled and cooled water in 1:1 and 1:2 ratio. The TSS content of the substrate was raised to 20° brix by adding cane sugar, followed by addition of yeast (1.25g/l). The must was treated with (KMS) potassium metabisulphite (0.05g/l) for the inhibition of other microorganisms. Clarified wine was evaluated for its quality. Wine was filled in glass bottles with crown cork, sealed, pasteurized at 82-88 ºC for 2 minutes and stored under ambient conditions for six months.

Determination of Wine yield:

After the fermentation, wine was decanted leaving the dead yeast cells and other residues at the bottom of the fermenting jar. The wine yield was expressed as percentage of weight of wine to the initial weight of fermenting substrate including fruit pulp, sugar and water.

$$\text{Wine Yield} = \frac{\text{Weight of wine obtained}}{\text{Initial Weight of fermenting substrate}} \times 100$$

pH:

pHofthe samples was measured by using the pH meter (Systronics, India). Standard buffer solutions of pH 4.0, 7.0 and 9.0 were used for calibration of pH meter.

Total soluble solids (TSS):

Total soluble solids were found out using a digital refractometer (range 0-320) and expressed as degree brix (^{0} Brix).

Titratable acidity:

Titratable acidity (TA) was calculated by using method described by AOAC (1998). For this first CO2 was removed by placing ca 25 mL sample in small Erlenmeyer flask, heating to incipient boiling and holding for 30 sec, swirled, and cooled. One mL of phenolphthalein indicator solution was added to 200 mL hot, boiled water in 500 mL wide-mouth Erlenmeyer flask. Neutralized to distinct pink. Five mL of degassed sample was added and titrated with 0.l NNaOH to same end point.

Then, Titratable acidity was calculated using formula given below by AOAC (1)

Titratable acidity = (mL NaOH x normality x 0.075 x 100)/5.

Alcohol content:

Specific gravity of the fermenting must and specific gravity of wine were determined separately. The alcohol content of wine was calculated by the following formula (13).

$$\text{Alcohol by volume \%} = \frac{\text{Original specific gravity- final specific gravity}}{7.36} \times 1000$$

Estimation of yeast population:

For determination of yeast population, Sabouraud's Dextrose Agar media was used using various dilution from 10^{-1} to 10^{-6}. One ml of each dilution was pipetted using a micropipette into a sterile Petridish. Approximately, 20 ml of the sterile, melted and cooled Sabouraud's Dextrose Agar (SDA) was poured into the Petridish and it was swirled. After solidification, it was kept for incubation at room temperature. Two Petri dishes were kept as replicate for each sample. The petriplates were incubated at room temperature for 3 to 5 days. The colonies developed were counted and expressed as cfu/ml of the sample (7).

Estimation of bacterial population:

Estimation of bacterial population was carried out using various 10^{-5} dilution on nutrient agar medium. Using a micropipette, one ml of 10^{-5} dilution was pipetted into a sterile petridish. Approximately 20 ml of the melted and cooled Nutrient Agar (NA) media was poured into the petridish and it was swirled. After solidification, it was kept for incubation at room temperature. Two petri dishes were kept as replicate for each sample. The Petri plates were incubated at room temperature for 48 hours. The colonies developed were counted and expressed as cfu/ml of sample (7).

Estimation of fungal population:
Fungal population was estimated using 10^{-3} dilution on Martin Rose Bengal Agar medium. One ml of 10^{-3} dilution was pipetted into a sterile Petridish using a micropipette. About 20 ml of the melted and cooled Martin Rose Bengal Agar (MRBA) media was poured into the Petridish and it was swirled. After solidification, it was kept for incubation at room temperature. Three Petri dishes were kept as replicate for each sample. The Petri plates were incubated at room temperature for 4 to 5 days. The colonies developed were counted and expressed as cfu/ml of the sample (7).

Estimation of Lactobacillus spp. Population:
Lactobacillus population was estimated using 10^{-4} dilution on MRS Agar medium. One ml of 10^{4} dilution was pipetted into a sterile petridish using a micropipette. Approximately 20 ml of the melted and MRS Agar medium was poured into Petri plates and it was swirled. After solidification, it was kept for incubation at room temperature. Two Petri plates were kept as replicate for each sample. The Petri plates were incubated at room temperature for 48 hours. The colonies developed were counted and expressed as cfu/ml of sample (7).

Sensory evaluation of banana wine:
Sensory quality of banana wine such as (colour/appearance, flavour, aroma, taste, after taste and overall acceptability) were analysed using a 9-point Hedonic scale (where 9 = like extremely and 1= dislike extremely) by 10 panellists (all men) aged 30–55 years) who were requested to analyse the samples in early morning separately on different days. They were familiar with wine consumption. Samples were served in clean transparent glasses (tumblers), which had been labelled with 3-digit random codes. Questionnaire sheet and mineral water for mouth ranging before each tasting were provided. Just before the analysis each panellist was familiarize with the product. The panellists were requested to read through the questionnaires and the superficial meaning of each attribute (colour/appearance, flavour, aroma, taste, after taste and overall acceptability) was explained to the panellists. The banana wine produced was presented to panellists for sensory analysis. The values presented here are average of panellist scores (6).

Results and Discussion: -

pH:
pH of all treatments showed an increase trend throughout storage. Wine stored in amber coloured bottles showed higher rate of increase compared to wine stored in plain bottles. After completion of storage, treatment W1 and W2 wine from Khozikodu recorded highest pH (4.21) and the lowest (3.72) was observed in W7 (wine from Alpan stored in amber coloured bottles). The rise in pH due to fall in the acidity may be due to precipitation of acids into salts. The increase in pH during storage of guava wine was reported by Shankar etal. (2).

Total soluble solids (^{0} Brix):
Total soluble solids of banana wine decreased throughout the storage period of six months. Wine stored in plain bottles showed higher rate of decrease compared to wine stored in amber coloured bottles. After six months of storage, treatment W1 and W2 (wine from Khozikodu stored in amber coloured and plain bottles respectively) recorded highest TSS (4.0) and the treatment W8 (wine from Alpan stored in plain bottles) had the lowest (3.65^0 Brix). Decrease in TSS during storage of wine may be due to conversion of sugar into alcohol by yeast. Decreasing trend of TSS during storage was reported in jamun wine (5).

Titratable acidity (%):
Titratable acidity of banana wine showed a decreasing trend during storage. Wine stored in plain bottles showed higher rate of decrease compared to wine stored in amber coloured bottles. After 6 months of storage, treatment W5 (wine from Alpan variety stored in amber coloured bottles) recorded the highest (0.54%) titratable acidity and the lowest was observed in W2 (wine from Khozikodu stored in plain bottles) (0.32%). Decline in titratable acidity may be due to precipitation of organic acids into their respective salts. Decrease in titratable acidity during storage of banana wine was reported by Brathwaite and Badrie. (10)

Volatile acidity:
Volatile acidity of banana wine showed a decreasing trend during storage for six months. Wine stored in plain bottles showed higher rate of decrease in volatile acidity compared to wine stored in amber coloured bottles. After 6 months of storage, treatment W4 and W6 (wine from Karpurchakra Keli and Palaykondan variety stored in plain

bottles respectively) recorded the highest (0.40%) volatile acidity and the lowest was observed in W2 (wine from Khozikodu stored in plain bottles) (0.32%).

Alcohol (%):
Alcohol content of banana wine revealed an increasing trend during storage. Wine stored in amber coloured bottles showed higher rate of increase compared to wine stored in plain bottles After 3 months of storage, treatment W8 (wine from Alpan variety stored in amber coloured bottles) recorded the highest alcohol content (6.58%) and the lowest (8.91%) was in W5 and W6 (wine from Palaykondan variety amber and plain bottles respectively). Nikhanj and Kocher (11), reported that there were no changes in alcohol production due to absence of yeast colonies which stopped further fermentation of guava wine.

Sensory evaluation:
Sensory evaluation analysis of banana wine (table 4.17) showed that in a hedonic scale of 9.0, the wine had good colour (7.1), flavour (6.2), aroma (6.1), taste (6.65), after taste (7.64) and overall acceptability (6.742). Wine produced from Aplan variety and stored in Amber coloured bottle showed highest acceptability (6.74). Wine from Palaykondan (W6) stored in plain bottle showed lowest acceptability score (5.5).

Yeast population (cfu/ml):
The yeast population was 0.3×103 cfu/ml in banana wine from various varieties after 1 month of storage. Thereafter, the yeast population declined and yeast could not be detected after 2nd and 3rd months of storage. The decline in yeast population during storage may be due to depletion of sugars, which serve as food for yeast. Similar decline in yeast population was also reported during storage of guava wine (11).

Microbial population (cfu/ml):
Bacteria, fungi and Lactobacillus could not be detected during storage of banana wine. The changes in pH, increase in alcohol content and anaerobic environment might have led to reduction in microbial count of wine. (8), noted that during the fermentation period of tendu (Diospyros melanoxylon) fruit wine, there was no detection of bacteria, fungi and other spoilage microorganisms except Saccharomyces cerevisiae. (4) Reported that during storage of banana wine there was no detection of bacterial and fungal population due to low pH and high alcohol content which led to decline in microbial population.

Table 1: Effect of storage conditions on pH, Total soluble solids (TSS) and Titratable acidity of banana wine

Treatment	P^H				Total soluble solids(^{0}Brix)				Titratable acidity(%)			
	Initial	2MAS	4MAS	6MAS	Initial	2MAS	4MAS	6MAS	Initial	2MAS	4MAS	6MAS
W1	4.12	4.14	4.18	4.21	4.55	4.52	4.45	4.38	0.37	0.38	0.35	0.34
W2		4.14	4.19	4.21		4.53	4.48	4.38		0.37	0.35	0.32
W3	3.75	3.80	3.95	3.76	4.63	4.55	4.45	4.30	0.51	0.48	0.46	0.46
W4		3.82	3.96	3.75		4.52	4.38	4.22		0.49	0.45	0.50
W5	3.82	3.88	3.89	3.92	4.40	4.40	4.32	4.20	0.63	0.60	0.55	0.54
W6		3.87	3.86	3.94		4.35	4.20	4.15		0.57	0.55	0.52
W7	3.60	3.58	3.69	3.72	3.85	3.90	3.85	3.72	0.48	0.50	0.47	0.45
W8		3.60	3.72	3.74		3.92	3.75	3.65		0.49	0.46	0.42

MAS-Months after storage
W1- (Khozikodu) wine stored in amber bottles W5- (Palaykondan) wine stored in amber bottles
W2- (Khozikodu) wine stored in plain bottles W6- (Palaykondan) wine stored in plain bottles
W3- (Karpurchakra keli) wine stored in amber bottles W7- (Alpan) wine stored in amber bottles
W4- (Karpurchakra keli) wine stored in plain bottles W8- (Alpan) wine stored in plain colouredbottles

Table 2: Effect of storage conditions Volatile acidity and Alcohol Percentage of banana wine

Treatment	Volatile Acidity (%)				Alcohol (%)			
	Initial	2MAS	4MAS	6MAS	Initial	2MAS	4MAS	6MAS
W1	0.36	0.37	0.34	0.34	6.23	6.23	6.32	6.38
W2		0.35	0.35	0.32		4.53	4.55	4.62
W3	0.41	0.40	0.41	0.38	4.86	4.92	4.90	4.92

W4		0.40	0.41	0.40		4.92	4.90	4.90
W5	0.43	0.42	0.41	0.39	4.34	4.45	4.50	4.51
W6		0.42	0.40	0.40		4.45	4.50	4.52
W7	0.40	0.38	0.38	0.39	6.38	6.50	6.52	6.58
W8		0.38	0.36	0.38		6.50	6.51	6.52

MAS-Months after storage

W1- (Khozikodu) wine stored in amber bottles W5- (Palaykondan) wine stored in amber bottles
W2- (Khozikodu) wine stored in plain bottleW6- (Palaykondan) wine stored in plain bottles
W3- (Karpurchakra keli) wine stored in amber bottle W7- (Alpan) wine stored in amber bottles
W4- (Karpurchakra keli) wine stored in plain bottlesW8- (Alpan) wine stored in plain bottles

Table 3: Effect of storage conditions on yeast, fungi and bacteria population of banana wine

Treatment	Yeast (10^3 CFU ml^{-1})				Fungi (10^3 CFU ml^{-1})				Bacteria (10^3 CFU ml^{-1})			
	Initial	2MAS	4MAS	6MAS	Initial	2MAS	4MAS	6MAS	Initial	2MAS	4MAS	6MAS
W1	3.0	a	a	a	0.30	a	A	a	0.10	a	A	a
W2		a	a	a		a	A	a		a	a	a
W3	3.5	a	a	a	0.28	a	a	a	0.20	a	a	a
W4		a	a	a		a	a	a		a	a	a
W5	3.2	a	a	a	0.28	a	a	a	0.25	a	a	A
W6		a	a	a		a	a	a		a	a	A
W7	3.6	a	a	a	a	a	a	a	0.15	a	a	A
W8		a	a	a		a	a	a		a	a	A

MAS-Months after storage a- Absent
W1- (Khozikodu) wine stored in amber bottles W5- (Palaykondan) wine stored in amber bottles
W2- (Khozikodu) wine stored bottles W6- (Palaykondan) wine stored in plain bottles
W3- (Karpurchakra keli) wine stored in amber bottles W7- (Alpan) wine stored in amber bottles
W4- (Karpurchakra keli) wine stored in plain bottles, W8- (Alpan) wine stored in plain bottles

Table 4 Sensory evaluation of the banana wine

Attribute / Variety	Colour	Flavour	Aroma	Taste	After Taste	Overall acceptability
W1	6.0	5.5	5.5	6.5	6.5	6.0
W2	6.0	5.5	5.5	6.5	6.5	6.0
W3	6.5	6.0	6.0	6.0	6.8	6.5
W4	6.5	6.0	6.0	6.0	6.8	6.5
W5	7.0	7.0	6.0	5.5	6.0	5.5
W6	7.0	6.0	5.5	6.5	6.0	5.0
W7	7.1	6.2	6.1	6.65	7.66	6.74
W8	7.1	6.2	6.1	6.65	7.66	6.74

n = 10, #Values are average of panellist scores
*9 = Like extremely 8 = Like very much 7 = Like moderately 6 = Like slightly 5 = Neither like nor dislike 4 = Dislike slightly 3 = Dislike moderately 2 = Dislike very much 1 = Dislike extremely

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