

Full Length Research Paper

Fermentation process for alcoholic beverage production from mahua (*Madhuca indica* J. F. Mel.) flowers

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Mahua flowers are rich in sugar (68-72%), in addition to a number of minerals and one of the most important raw materials for alcohol fermentation. The present investigation was for the development of a non-distilled alcoholic beverage from Mahua flowers. Eighteen (18) treatment combinations consisting of two temperatures (25 and 30°C), three pH (4.0, 4.5 and 5.0) and three period of fermentation (7, 14 and 21 days) were used in the fermentation conditions. The maximum yield of ethanol (9.51 %) occurred at 25°C with pH 4.5 after 14 days of fermentation of Mahua flower juice. The fermented non-distilled alcoholic beverage contained total sugar (8.83 mg/ml), reducing sugar (0.82 mg/ml), total soluble solids (6.37°Brix) titrable acidity (0.65 %), and volatile acidity (0.086%). Methanol was not detected at any stage of fermentation. The developed fermented alcoholic beverage had characteristic flavor and aroma of Mahua flowers with about 7 to 9% alcohol.

Key words: *Madhuca indica*, ethanol, reducing sugar, fermentation.

INTRODUCTION

Mahua (*Madhuca indica* J. F. Mel syn *Madhuca latifolia* Macb.) is a common tree in deciduous forests of India, quite prominent in states of Andhra Pradesh, Bihar, Gujarat, Karnataka, Madhya Pradesh., Orissa, Rajasthan, Uttar Pradesh and West Bengal. Mahua flowers are in dense fascicles near end of the branches having 1.5 cm long fleshy cream coloured corolla tube and are scented. Flowering period of Mahua is from the month of March to May. Flower induction starts from the top portion to lower branches and also from more illuminated part to shaded part of the tree. Flowers mature in about 32-35 days. One to two good flowering is

expected every three years, that is, it has an alternate bearing habit. Mahua flowers are rich in total sugars out of which maximum proportion is of reducing sugars. Sugars identified are sucrose, maltose, glucose, fructose, arabinose and rhamnose. When flowers are mature and ready to fall, there is maximum total sugar content in the flowers. Fructose is present in a greater proportion than glucose and in the ripe stage the quantities are almost equal. Sucrose increases in amount up to shedding of corollas and is latter converted into invert sugars. Mahua flowers are rich in total sugars (68-72%), out of which maximum proportion is of reducing sugars. Sugars

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identified are sucrose, maltose, glucose, fructose, arabinose and rhamnose. When flowers are mature and ready to fall, there is maximum total sugar content in the flowers (Sikarwar, 2002). However, in spite of being a rich source of nutrition, a major portion of dried Mahua flowers are being used in the preparation of country liquor (Patel and Naik, 2006). Wine is an alcoholic beverage typically made of fermented fruit juice and any fruit with good proportion of sugar may be used in producing wine and the resultant wine is normally named after the fruit. The type of wine to be produced dictates the fruit and strain of yeast to be involved (Idise, 2012). Therefore, the present investigation was carried out to study fermentation conditions for production of non-distilled alcoholic beverage that is wine from mahua flower juice extract.

MATERIALS AND METHODS

Mahua juice

The mahua flowers obtained were cleaned and dried in hot air oven at 60°C temperature for 5 h. 1 kg of mahua flowers was soaked in 1 L distilled water containing 1.5 g potassium meta bisulphite (KMS) for 12 h to prevent the growth of other contaminants. For preparation of juice from the flowers, additional water was added to facilitated the easy grinding. The mixture was ground with the help of electric mixture grinder and filtered through muslin cloth, stored in screw capped bottles and kept in a refrigerator at 4°C. The obtained juice had total soluble solids (TSS) 20°Brix, pH- 5.3 and volume about 3.5 L from 1 kg of dried mahua flowers.

Inoculum of yeast culture

The yeast strain obtained from the Department of Dairy and Food Microbiology, College of Dairy and Food Science Technology, Udaipur (*Saccharomyces cerevisiae*) was cultured on MGYB broth (Himedia, India) at 25°C for 48 h in a biological oxygen demand (BOD) incubator. The MGYB broth contained malt extract (0.75 g), peptone (1.25 g), yeast extract (0.75 g) and dextrose (5 g) in 250 ml distilled water. The yeast inoculum was had 4.8×10^4 cfu/ml.

Fermentation conditions

For optimization of fermentation conditions, the following treatment combinations were used: Temperature of 25 and 30°C; pH of 4.0, 4.5 and 5.0; period of fermentation of 7, 14 and 21 days. The pH of juice extract was adjusted at pH 4.0, 4.5 and 5.0 with help of citric acid. The yeast extract was added as a nitrogenous source at the rate of 0.1% (w/v) in the mahua juice. Each Erlenmeyer flask containing 100 ml of juice was inoculated with 1 ml (1% v/v) of 48 h old yeast culture under aseptic conditions. Then transferred in a BOD incubator for fermentation and maintained at 25 or 30°C for different periods of incubation. The samples were analysed after 7, 14 and 21 days of alcoholic fermentation. All experiments were conducted in triplicates and a control (un-inoculated) was also taken for each treatment.

Biochemical analysis of fermented samples

The total sugar was estimated by anthrone reagent method as

described by Hedge and Hofreiter (1962). Reducing sugars in samples were estimated by using dinitrosalicylic acid (DNS) reagent method as described by Miller (1972). The total soluble solids in the samples were determined with the help of hand refractometer (Erma) and expressed in terms of °Brix and pH of the extract was measured by using hand held pH meter (Eutech). Titrable acidity of the samples was determined by titration with 0.1 N sodium hydroxide and was expressed in terms of anhydrous citric acid (Ranganna, 1986). Volatile acidity of the fermented samples was determined after distillation. The distillate was titrated with 0.01 N sodium hydroxide and was expressed in terms of acetic acid (Ranganna, 1986). The ethanol in the fermented sample was determined by using the specific gravity method (AOAC, 2000). The methanol in the fermented sample was determined with the help of quantitative colorimetric micro determination method (Boos et al., 1948).

RESULTS AND DISCUSSION

The optimization study was done by using different combination of temperature, pH and duration of fermentation. The alcoholic beverage obtained after fermentation was analysed for total sugar, reducing sugar, total soluble solids, titrable acidity, volatile acidity and ethanol. In the present investigation, the total sugar were found maximum (96.47 mg/ml) at 25°C with pH 5.0 after seven days of fermentation whereas, minimum total sugar (0.45 mg/ml) were found at 30°C with pH 4.5, after 21 days of fermentation (Table 1). The total sugar content decrease with the increasing period of fermentation in all the treatments irrespective of pH and temperature. Yeast cells require carbon and nitrogen sources for their growth and development. During fermentation, sugar is utilized for energy production and ethanol is produced as a by-product of the fermentation process.

With increase in fermentation period, there was decrease in total sugar content because sugar was being utilized for ethanol production. At higher temperature the metabolic reaction of yeast cells increase which resulted into faster decrease in the total sugar content of the mahua flower juice extract during the fermentation studies. Rivera-Espinoza et al. (2005) have reported that the fermentation of cane sugar juice at 30°C temperature is faster than that at 25 and 28°C temperature. Similar observations were reported by Singh and Kaur (2009) in case of litchi wine production where total sugar decreased from 85.25 to 3.5% (w/v) after five days fermentation. Yan et al. (2012) observed that many factors can affect the ethanol content and volatile acid content of blueberry wine and suggested that the maximum ethanol content and minimum volatile acid production of blueberry wine fermentation with *Saccharomyces cerevisiae* AS2.316 commercial wine yeast could reach 7.63% and 0.34 g l^{-1} under the optimal condition of temperature, 22.65°C; pH, 3.53; inoculum size, 7.37%.

Total reducing sugar content of fermented alcoholic beverage decreased during fermentation period. The reducing sugar were found maximum (57.68 mg/ml) at

Table 1. Total sugars (mg/ml) during fermentation of Mahua flowers extract.

Fermentation medium	pH of fermentation medium	25°C			30°C			Mean
		Period of incubation (days)						
		7	14	21	7	14	21	
Sample	4.0	92.62	8.77	1.10	79.17	12.71	0.46	32.47
Control	4.0	219.31	218.35	217.63	218.94	219.31	218.15	218.61
Simple	4.5	88.17	8.83	0.74	65.75	11.53	0.45	29.25
Control	4.5	218.15	217.53	217.33	216.84	216.44	216.25	217.09
Sample	5.0	96.47	9.60	0.60	79.97	10.95	0.49	33.01
Control	5.0	217.13	216.54	215.54	218.22	217.92	217.03	217.07
Mean		155.31	113.27	108.82	146.48	114.81	108.80	124.58
Interaction		SEM±			CD (0.0 5)			
Temperature		0.49			1.39			
Days		0.60			1.70			
pH		0.60			1.70			
Temp.x Daysx pH		1.48			NS*			

*NS stands for non-significant.

Table 2. Reducing sugars (mg/ml) during fermentation of Mahua flowers extract.

Fermentation medium	pH of fermentation medium	25°C			30°C			Mean
		Period of incubation (days)						
		7	14	21	7	14	21	
Sample	4.0	57.68	0.90	0.48	53.60	0.86	0.46	18.99
Control	4.0	187.56	186.53	185.66	185.37	185.51	184.47	185.85
Simple	4.5	43.96	0.82	0.46	52.05	0.75	0.43	16.41
Control	4.5	184.49	184.05	183.18	184.91	183.03	182.30	183.66
Sample	5.0	49.80	0.84	0.46	52.32	0.71	0.44	17.43
Control	5.0	184.64	184.06	184.20	183.91	182.87	181.68	183.56
Mean		118.02	92.87	92.41	118.69	92.29	91.63	100.98
Interaction		SEM±			CD (0.0 5)			
Temperature		0.44			1.26			
Days		0.55			1.55			
pH		0.55			1.55			
Temperaturex Days x pH		1.34			NS*			

*NS stands for non-significant.

25°C with pH 5.0 after seven days of fermentation. The reducing sugar content decreased gradually with the increasing period of fermentation. After 21 days of fermentation, the lowest reducing sugar (0.43 mg/ml) was recorded at 30°C with pH 4.5 (Table 2). Reducing sugar is a fermentable sugar and decreases with increasing period of fermentation. Reducing sugar is most important sugar for fermentation as it is easy to metabolize by yeast. Similarly, Reddy and Reddy (2009) reported that reducing sugar was decreased (from 185 to 2.0 g/litre) after alcoholic fermentation of mango juice. Yadav et al. (2009) observed reducing sugar decrease up to 0.08% in mahua wine at 30°C temperature after 15 days of fer-

mentation. Chowdhury and Roy (2007) reported that the reducing sugar content decrease from an initial value of 6.48 ± 0.06 g/100 ml in jamun must to 0.49 ± 0.04 g/100 ml in wine prepared from jamun.

The total soluble solids content of fermented alcoholic beverage decreased with increase of fermentation period up to the end of experimentation. The highest TSS content (7.87°Brix) was observed at 25°C with pH 5.0 after seven days of fermentation and lowest TSS content (5.50°Brix) were found at 30°C with pH 5.0 after 21 days of fermentation (Table 3). The decrease in TSS content of mahua flower alcoholic beverage indicates the utilisation of sugar present in the mahua flower juice

Table 3. Total soluble solids ($^{\circ}$ Brix) during fermentation of Mahua flowers extract.

Fermentation medium	pH of fermentation medium	25°C			30°C			Mean
		Period of incubation (days)						
		7	14	21	7	14	21	
Sample	4.0	7.27	6.37	6.07	6.97	6.23	5.80	6.45
Control	4.0	19.87	19.73	19.67	19.90	19.73	19.60	19.75
Simple	4.5	7.60	6.37	6.00	7.07	6.23	5.67	6.49
Control	4.5	19.80	19.77	19.67	19.93	19.87	19.73	19.79
Sample	5.0	7.87	6.63	6.00	7.20	6.27	5.50	6.58
Control	5.0	19.80	19.77	19.63	19.87	19.83	19.60	19.75
Mean		13.70	13.11	12.84	13.49	13.03	12.65	13.14
Interaction		SEM \pm			CD (0.0 5)			
Temperature		0.01			0.04			
Days		0.02			0.06			
pH		0.01			0.06			
Temperature \times Days \times pH		0.05			NS*			

*NS stands for non-significant.

extract during the fermentation process. It is obvious from the data that, with the increased temperature, the rate of fermentation also increased which in turn decreased the TSS of mahua flower extract during the fermentation. Similarly, Ukwuru and Awah (2013) observed that the purified yeasts from palm wine showed highly viable cells and good metabolic activity during grape must fermentation. Grape must fermentation resulted in increase in temperature (28 to 32°C) and reduction in pH (4.3 to 3.1) and total solid concentration in the wines decreased consistently during fermentation (21 to 5%). Titratable acidity increased during fermentation from 0.44 to 0.82%. Akubor et al. (2003) observed the decrease in TSS of banana juice from 18 to 4.8 $^{\circ}$ Brix at the end of 14 days fermentation at 30 \pm 2°C temperature. The trend of TSS decrease during various conditions of mahua extract fermentation is similar to those reported by Chowdhury and Ray (2007) in jamun wine fermentation process. Similarly, Ezeronye (2004) have reported the reduction of TSS in cashew apple juice from 24 to 6.0 $^{\circ}$ Brix after 14 days fermentation at 20°C during cashew apple wine fermentation.

The titratable acidity of fermented alcoholic beverage exhibited increasing trends till the end of experimentation in all the treatments. There was positive effect of fermentation and treatment combinations on titratable acidity during entire fermentation period as shown in Table 4. After fermentation, maximum acidity (1.11%) was observed at 30°C with pH 5.0 after 21 days of fermentation, whereas, minimum titratable acidity (0.55%) was found at 25°C with pH 4.5 after seven days of fermentation. Similar observations were made by Chowdhery and Roy (2007) when they reported an increase in titratable acidity (from 0.51 to 3.30%) during the alcoholic fermentation. However, Vaidya et al. (2009)

reported decrease in titratable acidity (from 1.07 to 0.52%) after fermentation of kiwi fruit juice into wine at 22 \pm 1°C. Titratable acidity is an important characteristic of wines and it depends on the biochemical composition of fruit juice used in the alcoholic fermentation and process parameters of fermentation. The titratable acidity of fruit wines vary between 0.5-1.0% ((Joshi, 1998).

The change in volatile acidity of fermented alcoholic beverage during fermentation under different treatment combinations was assayed for quality of alcoholic beverage. The volatile acidity of fermented alcoholic beverage exhibited increasing trends till the end of experimentation in all the condition of fermentation. The maximum volatile acidity (0.134%) was observed at 30°C with pH 5.0 after 21 days of fermentation, whereas, minimum volatile acidity (0.070%) was found at 25°C with pH 4.5 after seven days of fermentation (Table 5). The concentration of total volatile compounds increased during fermentation. Volatile acidity may result from the coupled oxidation of wine phenolics to yield peroxide which in turn oxidized ethanol to acetaldehyde and subsequently to acetic acid (Zoecklein et al., 1995). Volatile acidity of strawberry fruit wine may vary from 0.027-0.030% acetic acid equivalent (Joshi et al., 2005; Joshi et al., 2006).

The ethanol content of fermented alcoholic beverage was estimated at various intervals of fermentation under different treatment combinations. The ethanol content of fermented alcoholic beverage increased with advancement of fermentation period in all the treatments. The treatments had significant effect on ethanol content of fermented alcoholic beverage during entire fermentation periods (Table 4). The maximum ethanol (9.51%) content was observed at 25°C with pH 4.5 after 14 days of fermentation and minimum ethanol (6.70%) was found

Table 4. Titrable acidity (%) during fermentation of mahua flowers extract.

Fermentation medium	pH of fermentation medium	25°C			30°C			Mean
		Period of incubation (days)						
		7	14	21	7	14	21	
Sample	4.0	0.58	0.69	0.80	0.68	0.75	0.94	0.74
Control	4.0	0.39	0.41	0.42	0.39	0.41	0.42	0.41
Sample	4.5	0.49	0.65	0.76	0.65	0.85	1.01	0.74
Control	4.5	0.35	0.42	0.46	0.38	0.41	0.45	0.41
Sample	5.0	0.55	0.78	1.00	0.65	0.89	1.11	0.83
Control	5.0	0.34	0.42	0.45	0.35	0.46	0.42	0.41
Mean		0.45	0.56	0.65	0.51	0.63	0.73	0.59
Interaction		SEM±			CD (0.0 5)			
Temperature		0.006			0.01			
Days		0.008			0.02			
pH		0.008			0.02			
Temperature x Days x pH		0.019			NS*			

*NS stands for non-significant.

Table 5. Volatile acidity (%) during fermentation of mahua flowers extract.

Fermentation medium	pH of fermentation medium	25°C			30°C			Mean
		Period of incubation (days)						
		7	14	21	7	14	21	
Sample	4.0	0.072	0.088	0.108	0.074	0.100	0.106	0.091
Sample	4.0	0.070	0.086	0.108	0.078	0.108	0.126	0.096
Sample	4.5	0.086	0.104	0.130	0.092	0.124	0.134	0.112
Mean		0.076	0.093	0.115	0.081	0.111	0.122	0.100
Interaction		SEM±			CD (0.0 5)			
Temperature		0.002			0.013			
Days		0.002			0.005			
pH		0.002			0.005			
Temperature x Days x pH		0.005			NS*			

*NS stands for non-significant.

at 25°C with pH 4.0 after seven days of fermentation. There was an increase in ethanol content up to 14 days of fermentation and it decreased in all the treatments combinations after 21 days of fermentation (Table 6). Thornton and Rodriguez (1996) reported that juices should be fermented at lower temperature (often 15°C) to retain their fruity character. Chowdhury and Ray (2007) made red wine from jamun (*Syzgium cumini* L.) fruit with low alcohol (6%) concentration after six days of fermentation at 32 ± 2°C. They reported that low alcohol content in jamun wine was probably due to low TSS (16.5 °Brix) in jamun must in comparison to grape must TSS (usually 22 to 24°Brix) which yields wine with 8 to 10% alcohol. The percent ethanol obtained in present investigation is in accordance with several workers who reported that wine made from fruit juice fermentation

contains about 8 to 10 (v/v)% ethanol (Yadav et al., 2009). Similarly, Reddy and Reddy (2005) observed that pH 5.0 and temperature 30°C were optimum for highest ethanol production (7-8%) in case of mango wine. However, Yadav et al. (2009) observed that mahua wine fermented at 16°C had higher content of alcohol (9.9%) compared to that at 20 and 25°C after 15 days of fermentation. This difference in ethanol content may be attributed to nutritional composition of mahua flower and yeast strain used for fermentation. Soni et al. (2009) had reported highest alcohol content (10%) of amla (*Amblica officinalis*) wine at 25 and 30°C but fermentation efficiency decreased at higher than 30°C. Most of the yeast strains grow best at a temperature less than 35°C. Changes in membrane fluidity of the mesophilic yeast lead to a retarded or no growth at higher temperature

Table 6. Ethanol (%) during fermentation of mahua flowers extract.

Fermentation medium	pH of fermentation medium	25°C			30°C			Mean
		Period of incubation (days)						
		7	14	21	7	14	21	
Sample	4.0	6.70	9.40	7.20	7.25	8.44	6.94	7.65
Sample	4.0	7.51	9.51	7.53	8.09	9.29	7.46	8.23
Sample	4.5	6.91	9.16	6.96	7.64	9.18	6.85	7.78
Mean		7.04	9.36	7.23	7.66	8.97	7.08	7.89
Interaction		Sem±			CD (0.0 5)			
Temperature		0.21			0.61			
Days		0.08			0.20			
pH		0.08			0.20			
Temperature x Daysx pH		0.21			NS*			

*NS stands for non-significant.

(Banat et al., 1998). Methanol is a harmful content of alcoholic beverage. It is always hazardous for human health. In our study, the methanol content was not detected at any stage of fermentation process. Similarly, Rivera-Espinoza et al. (2005) reported that methanol was not detected in the course of alcoholic fermentation from sugarcane juice. Okunowo and Osuntoki (2007) reported that fermentation of the orange juice by *S. cerevisiae* from yam and *S. cerevisiae* from sugarcane molasses resulted in products with different concentrations of alcohol types despite the fact that the fermenting organisms are of the same species. This indicates that the source of the yeast may influence the alcohol profile of the wine produced. It is therefore concluded that the source of the yeast is thus an important factor in the determination of the quality of the wine.

Though the level of ethanol production was higher after 14 days of fermentation at both temperature, seven days of incubation at 25°C was selected as optimized condition for fermentation to retain the characteristics aroma of mahua flowers because at 30°C there was increase in total titrable acidity.

It may be concluded that elaboration of alcoholic beverage with acceptable characteristics of wine that may be produced by using mahua flower juice extract as a substrate, is technically feasible and a good alternative use for raw material. It is our hope that these optimized conditions can be an incentive for an eventual commercial production of non-distilled alcoholic beverage from mahua flowers in an economical way.

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