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Fermentative production of an antioxidant rich jamun vinegar by a packed bed fermentation process

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ABSTRACT

Jamun a tropical fruit rich in anthocyanins possesses an array of medicinal properties arising from its phenols, glycosides and alkaloids. The fermentation of jamun juice by *Saccharomyces cerevisiae* MTCC 11815 produced 7.8% (v/v) of ethanol in 48 h with a fermentation efficiency of 98.6%. The vinegar produced by batch and packed bed fermentation at 25 L scale revealed a volatile acidity of 5.8% (w/v) and 6.2% (w/v) in 28 and 11 days, respectively. The supplementation of seed and pulp powder improved the total phenols in the vinegar. The supplementation of seed and pulp powder further improved the total phenols in the vinegar from 140.4 to 187.4 mg/100 ml. Likewise the free radical scavenging activities of vinegar were also improved with decreased EC50 values from 38.64 to 24.79 and higher AEAC µM values from 0.44 to 0.69, respectively in the two types of jamun vinegar.

Key words: *Syzygium cumini*, *Acetobacter aceti,* free radical scavenging, *Saccharomyces cerevisiae*, vinegar.

INTRODUCTION

Natural vinegar is a known panacea for a number of chronic diseases like diabetes, rheumatism, cardiovascular and even neurological problems. These properties emanate from the potential of vinegar to scavenge free radicals owing to the presence of fruit and vegetable borne antioxidants like phenolics, flavonoids, carotene, vitamins etc. Moreover, fruits and vegetables, the substrates for vinegar fermentation are seasonal while their vinegar retain the naturality of fruit extracts that can be used all round the year for their benefits.

Vinegar is prepared from fruits and vegetables and has been reported from apple, cherry, mango, sugarcane, plum, strawberry, pineapple etc. Jamun *(Syzygium cumini*) is always appreciated for the color, flavor and taste of its fruit. Apart from oxalic and tannic acids and certain alkaloids, jamun fruit is rich in carbohydrates, minerals and vitamins and is known for its antioxidant as well as medicinal benefits (Chowdhury and Ray, 4).

A batch scale technology for sugarcane vinegar fermentation at 50L scale was developed in our laboratory which took 25-28 days for producing vinegar (Kocher *et al.,* 9). Further, immobilized cells are reported not only to reduce fermentation time (5-7 days, Kocher *et al.,* 8), but also help in early clarification of vinegar. Cells adsorbed on carriers such as hollow fibres, polyurethane, wood shavings, corn cobs and bagasse have been used for immobilization of *Acetobacter* cells and are found to have better fermentation efficiency. Earlier, the wood shavings immobilized cells were found to be better at 25 L scale, wherein 10 successive rounds of sugarcane vinegar produced a consistent minimum volatile acidity of 4 % (w/v) (Kumar and Kocher, 10). The present study was thus conducted to validate the packed bed jamun vinegar fermentation process at 25 L scale along with determination of its antioxidant potential.

MATERIALS AND METHODS

Jamun was procured from Regional Research Station, Bhaduargarh, Patiala. The cultures used in the study were the fermenting yeast *Saccharomyces cerevisiae* MTCC 11815 that was cultivated on glucose yeast extract broth and the vinegar bacterium *Acetobacter aceti* which was grown in tryptone broth (Kumar and Kocher, 10).

One quintal of jamun berries were boiled with 50L of water to obtain 75L of jamun juice with a brix of 6.0±2.0˚B, pH 4.5±0.5 and acidity of 0.6±0.1. Brix–acid ratio of jamun juice was adjusted in the desirable range (29-40) by using sugar (raising Brix to 13.0°B). The latter was pasteurized at 80˚C in a covered steel container for 20 minutes and allowed to cool for overnight. The juice was supplemented with 0.15% (w/v) of DAHP and inoculated with freshly prepared (in jaggary solution @ 150g/l) 24h old inoculum of *S. cerevisiae* MTCC 11815 @ 7.5% (v/v) followed by its incubation at 28±2°C (Kocher *et al.,* 8), till brix (measured by digital refractometer and glass brixometer) decreased to zero. Periodic samples

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(12 hourly) were taken aseptically and analysed for brix, ethanol (Caputi and Wright, 2) and pH (hand pH meter, Hanna make).

The ethanol obtained by fermentation of jamun juice was fermented by both batch and packed bed fermentation. In batch fermentation, the acetic acid fermentation of jamun ethanol was carried out at 25L working volume. The fermentation was performed by inoculating freshly prepared 7.5% (w/v) 48h old inoculum of *A. aceti* (in jaggary solution @ 15g/l) and 10% (v/v) of mother vinegar so as to have 1% initial acidity followed by incubation at 28±2°C (Kocher *et al.,* 8). A total of five sequential fermentation cycles were carried out with 3 replicates of each. The fermenter was incubated at 28±2°C and used to measure volatile acidity (AOAC, 1) and residual ethanol as discussed earlier. In packed bed fermentation, the acetic acid fermentation of jamun ethanol was carried under already standardized conditions {15mm *Melona grandis* (Mirindi} wood shavings (in the ratio 2:1 with culture) for 15h with 0.2% DAHP supplementation) at 25L scale (Kumar and Kocher, 10). Each column was packed with *A. aceti* cells adsorbed wood shavings and charged with jamun ethanol mixed with mother vinegar in a ratio of 3:2, so as to have an initial acidity of 2% (w/v). A total of five sequential fermentation cycles were carried out with 3 replicates of each. The fermenters were incubated at 28±2°C and periodic samples were used to measure volatile acidity (AOAC, 1) and residual alcohol as discussed earlier. The effect of supplementation of jamun seed and pulp powders on packed bed fermentation was also studied by adding these @ 286.4 and 300 mg/100 ml, respectively (unpublished data). The results of fermentation experiments were analysed statistically using CPCS1.

Free radical scavenging activity: The *in vitro* antioxidant potential of juice, wine and vinegar from jamun was estimated as total free radical scavenging activity by DPPH method (Sanchez-Moreno *et al.,* 13). The effective concentration (EC_{50}) and ascorbic acid equivalent antioxidant capacity (AEAC) values of fermented vinegars were also calculated by the method of Shimamura *et al.* (14) as per following formulae:

Per cent inhibition = {O.D (Control) – O.D (Test sample) / O.D (Control)} × 100.

AEAC = EC_{50} of ascorbic acid (µM) / EC_{50} of samples (µM)

Anthocyanins and Total phenols from juice, wine and vinegar of jamun were estimated by the methods of Zoecklein (15) and Malik and Singh (12), respectively.

RESULTS AND DISCUSSION

The ethanolic fermentation of jamun juice (13±0.5°B) using *S. cerevisiae* MTCC 11815 (5% w/v) was complete in 48h with an ethanol production of 7.8% (v/v), a fermentation efficiency of 98.6% and an ethanol yield of 0.504 gg^{-1} (Table 1). Besides, the pH of the juice decreased from 4.5 to 4.0 in 48h of fermentation due to production of carbonic acid and other minor acids in the fermenting juice (Kocher *et al.,* 9). Chowdhury and Ray (3) reported that wine prepared from jamun was sparkling red in colour, acidic in taste and having low alcohol (6%). Maximum ethyl alcohol of 7.92 % and jamun wine recovery of 86.15 per cent was recorded from an initial 24°B by Lokesh *et al.* (11).

The optimized conditions for sugarcane vinegar production were validated for jamun vinegar production at 25L scale (Kumar and Kocher, 10). It resulted in production of jamun vinegar of an average final acidity of 6.2% (w/v) in 11 days at 25 L scale which is again within the limit prescribed by FSSAI (Gaur, 5) as well as higher than batch fermentation (Table 2). Earlier, ethanol produced from jamun juice was subjected to acetic acid fermentation under already optimized conditions for sugarcane vinegar production at a scale of 50 L in our laboratory (Kocher

Table 1. Ethanolic fermentation of jamun juice by *S. cerevisiae* MTCC 11815.

Fermentation Period (h)	TSS (Brix)	Total Sugars (g/100ml)	Reducing Sugars (g/100ml)	Ethanol (% v/v)	pH
0	13.0±0.20	12.2±0.18	11.7±0.25	0.00	4.5±0.5
12	10.5±0.15	9.7±0.23	9.1±0.18	1.52±0.05	4.4±0.1
24	4.0±0.20	3.4±0.15	2.6±0.28	5.7±0.05	4.4±0.0
36	1.5±0.15	0.8±0.08	0.3±0.15	7.17±0.07	4.2±0.05
48	0.0	0.1±0.05	0.0±0.0	7.7±0.05	4.0±0.0
Fermentation Efficiency (%)			98.6		
Ethanol Yield (g/g)			0.504		

Fermentation Conditions: Scale of fermentation; 50L, Temperature; 28±2ºC, Inoculum; 5% (v/v), Initial inoculum size; 5.0×10^5, Final inoculum size; 8.8×10^8.

Table 2. Comparative fermentation of jamun juice under Batch and packed bed conditions by *Acetobacter aceti* AC1.

Fermentation cycles	Batch*			Packed bed*		
	Initial	Final	Days	Initial	Final	Days
1	1.1±0.60	5.2±0.28	30±1.65	2.0±0.11	6.0±0.33	14±0.77
2	2.0±0.11	5.8±0.31	25±1.37	2.1±0.11	6.5±0.35	11±0.60
3	1.8±0.09	6.1±0.33	28±1.54	2.2±0.12	6.4±0.35	12±0.66
4	2.2±0.12	6.3±0.34	29±1.59	1.9±0.10	5.9±0.32	10±0.55
5	2.1±0.11	5.7±0.31	27±1.48	2.1±0.11	6.1±0.33	8±0.44
Mean±SD	1.8±0.10	5.8±0.32	28.0±1.52	2.0±0.11	6.2±0.33	11±0.60
*$CD_{(5\%)}$			0.081			

Fermentation conditions: Temperature; 28±2ºC, Initial alcohol; 8.0% (v/v), Residual alcohol; 0.3±0.4% (v/v); Scale of fermentation, 25L.

et al., 8). A total of five sequential fermentation cycles (25 L working volume, each) were carried out with 3 replicates of each wherein an average final acidity of 5.8 % (w/v) was obtained in 28 days (Table 2) well within the limits prescribed by FSSAI (Gaur, 5). Earlier, De Ory *et al.* (4) reported vinegar production in 225L pilot plant producing high quality vinegar with a yield of 100%. Vinegar production in a low-cost plastic fermenter by recycling two-thirds of the volume with freshly fermented brewed ethanol was earlier reported by us to produce a high-acidity sugarcane vinegar in a shorter period of 8 days at 50 L scale (Kocher *et al.,* 8).

There was a significant increase in phenols and tannins with supplementation of seed and pulp powders while days of fermentation, acetification rate and anthocyanins didn't reveal significant changes (Table 3). It has been reported earlier that jamun seeds and pulp are rich sources of phenols, anthocyanins and tannins (Jebitta and Allwin, 6)

The total free radical scavenging activities in terms of DPPH (Table 4) for jamun juice, ethanol and vinegar were tested that revealed EC_{50} values of 26.9, 17.7, 38.64 and 24.79 µM and AEAC values of 0.63, 0.96, 0.44 and 0.69, respectively. Further, EC_{50} of ascorbic acid taken as positive control was 17.2 µM. It has been earlier reported that antioxidant activity is high in jamun seeds and varies greatly among the other parts of jamun fruit. In one of the reports, antioxidant activity was found to increase by 20.52% after incorporation of jamun pulp at 20 per cent in pear juice (Kapoor and Ranote, 7). The high antioxidant properties of jamun is due to phytochemical antioxidants that include ascorbic acid (vitamin C), tocopherols, and tocotrienols (Vitamin E). Besides, jamun is also rich in nutraceuticals and biologically active compounds such as vitamins, dietary fibers and minerals. In the present study, an analysis of anthocyanins and phenols in Jamun juice, wine and vinegar was conducted that revealed anthocyanins and total phenols of 23.8, 22.4, 18.9 and 20.4 mg/100ml and 142.3, 148.7, 140.4 and 187.4 mg/100ml respectively (Table 4). Earlier Jebitta and Allwin (6) reported that even freeze dried samples of jamun possessed 105.7 mg/g of total flavonoids and 7.25 mg/g of anthocyanins with 13.99 mg/g of total phenols. Hence, due to retention of phenolics and anthocyanins, the antioxidant properties of vinegar were preserved.

To conclude, the jamun vinegar production technology by batch and packed bed cells was successfully validated at 25L scale and was found

Table 3. Effect of blending jamun seed powder and pulp powder on acetic acid fermentation of jamun juice under packed bed conditions by *A. aceti* AC1.

Measurable parameters	Acetic acid fermentation Treatments	
	Control (Unsupplemented)	Seed and pulp powder blended
Number of days	11±0.60	11.5±0.48
Volatile acidity (g%)	6.2±0.33	6.1±0.98
Acetification rate (g/day)	0.56±0.13	0.53±0.24
Total phenols (mg/100ml)	140.4±7.60	187.4±5.82
Anthocyanins (mg/100 ml)	18.9±2.75	20.4±1.78
Tannins (mg/100ml)	1.63±0.058[a]	1.93 ±0.026[b]

Seed powder and pulp powder were supplemented @ 286 and 300 mg/100 ml, respectively.

Table 4. DPPH scavenging activities, anthocyanin content and total Phenols in jamun juice, ethanol and vinegar.

Samples	Concentration (µM)	% DPPH scavenging activity	EC_{50} Value (µM)	AEAC (µM)	Anthocyanins (mg/100ml)	Total Phenols (mg/100ml)
Jamun juice	10	36.7	26.98	0.63	23.8	142.3
	30	53.3				
	70	78.5				
	100	93.4				
Jamun wine	10	41.6	17.77	0.96	22.4	148.7
	30	61.8				
	70	84.3				
	100	96.7				
Jamun vinegar	10	30.8	38.64	0.44	18.9	140.4
	30	54.7				
	70	75.8				
	100	92.7				
Jamun vinegar (Blended)	10	34.7	24.79	0.69	20.4	187.4
	30	59.1				
	70	80.1				
	100	94.3				

EC_{50} value of ascorbic acid w.r.t. DPPH were 17.2 µM and AEAC of 1.0

to have antioxidant potential equivalent to juice thus demonstrating that vinegar retained antioxidant properties, anthocyanin content and total phenols of juice. Since vinegar has a higher shelf life, it can be a free radical quenching source even during off season of substrate for which it is prepared. Further, the natural vinegar production optimized in the present study was economical and an industrially viable technology.

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