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ORIGINAL ARTICLE

Production of tea vinegar by batch and semicontinuous fermentation

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Abstract The fermented tea vinegar combines the beneficial properties of tea and vinegar. The complete fermentation takes 4 to 5 weeks in a batch culture and thus can be shortened by semi continuous/ continuous fermentation using immobilized bacterial cells. In the present study, alcoholic fermentation of 1.0 and 1.5% tea infusions using *Saccharomyces cerevisae* G was carried out that resulted in 84.3 and 84.8% fermentation efficiency (FE) respectively. The batch vinegar fermentation of these wines with *Acetobacter aceti* NRRL 746 at the initial 1% acidity yielded 4.5 and 4.7% volatile acidity with 71.4 and 73% FE in 24 days. The semi continuous fermentation using sugarcane bagasse adsorbeded *A.aceti* cells produced 4.4% of volatile acidity from 1.5% tea wine (8.9% ethanol and 1.0% acidity), in 9 flow cycles, each of 4 h duration in a fermentation column at 50 ml/h. In the scale up process, 600 ml 1.5% tea wine produced 4.3% volatile acidity at 42.7% FE in 9 flow cycles of 12 h duration each.

Keywords Tea wine · Vinegar · Volatile acidity · Semi continuous fermentation · Immobilization

Introduction

The extract, infusion or decoction of tea, especially black tea is the most liked beverage of the world. Daily per capita consumption of tea is about 100 ml and tea consumption is ranked second only to water and much ahead of hard and soft drinks (Hui 1992, Wrobel and Urbina 2000). The black tea is produced from fermented, withered, dried leaves of *Camellia sinensis* and has 2 types-Orthodox (rolled whole leaf) and CTC (crush, tear, curl). Tea leaves contain upto 36% of methylxanthines (caffeine) and polyphenols (having antioxidant properties) besides having good quantities of amino acids, proteins, carbohydrates and minerals essential to human health (Horzic et al. 2009). The tea vinegar which is a product of dual fermentation of tea extract, can be considered a conglomerate of exclusive beneficial properties of tea and vinegar. The tea extract is rich in proteins, amino acids, lipids, volatiles and all powerful antioxidant flavonoids. The vinegar offers cure or control of bone fractures, constipation, cold, cough, dandruff, diarrhoea, hypothermia, toothache etc. (Johnston 2006). Rice vinegar is believed to prolong ageing (Yamagishi et al. 1998). A fermented tea decoction 'Kombucha' prepared from yeast and acetic acid bacteria is a designated health drink (Guttapadu et al. 2000). The black tea extract fermented by yeasts accumulates vitamins A, C and B-complex, possesses nutritional and therapeutic properties (Chand and Gopal 2005) and enhances its own shelf life (Liyanage et al. 1988). Notwithstanding these larger benefits, information on the development of fermented tea beverages, particularly tea vinegar is obscure.

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Materials and methods

Tea brew Brook Bond black CTC tea (1.0 and 1.5% *w*/*v*) was brewed for 3 min in near boiling water and the extract was sieve (140 mesh) filtered. The tea extract, while still hot was sweetened with sucrose (commercial grade cane sugar) to have desired total soluble solids (TSS) of 15–25°B. It was cooled to room temperature, pH adjusted to 4.5, dispensed in sterilized glass bottles (550 ml capacity and loosely plugged with cotton wool).

Fermentation The bottles having 400 ml brew were inoculated with 24 h old broth culture of *Saccharomyces cerevisiae* G at 5% (*v*/*v*) equivalent to approx. 10^6 cells/ml

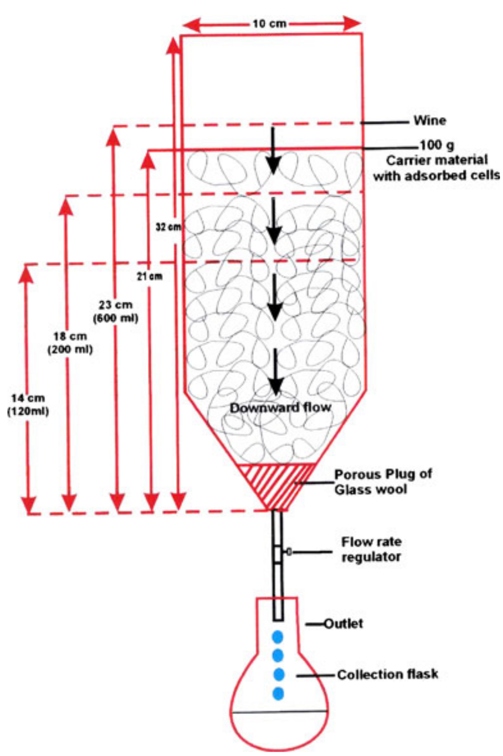
Table 1 Effect of sugar concentration on ethanol fermentation of tea infusion

Tea conc., %	Sugar added, %	TSS, °B		Sugars, %		Ethanol produced, % v/v	FE, %	Final pH
		Initial	Final	Initial	Residual			
1.0	15.0	14.0	1.4	13.2	0.8	6.1±0.28	72.1	2.92
	20.0	18.6	3.5	16.0	1.0	8.6±0.57	84.3	2.97
	25.0	23.4	5.2	23.1	1.8	12.0±0.99	81.1	3.07
1.5	15.0	13.8	1.2	12.8	0.6	6.2±0.37	75.6	3.10
	20.0	18.4	3.0	16.4	1.0	8.9±0.71	84.8	3.16
	25.0	23.2	4.3	22.8	1.2	12.2±0.85	83.6	3.21
CD (0.05), (*n*=3)						0.21		

TSS: Total Soluble Solids, FE: Fermentation efficiency

and incubated at 28±1 °C under still conditions. The fermentation reaction continued for 2 to 4 days until TSS dropped to constant lowest brix that was different in different treatments. The tea wine so obtained was clarified by sedimentation at <10 °C for 7–10 days and siphoned off. The vinegar fermentation was carried out in lots of 200 ml wine in glass bottles as above at 30±1 °C using 5% (*v/v*) 36 h old inoculum broth of *Acetobacter aceti* NRRL 746 and ≥5% mother vinegar as per different treatments. The batch fermentation proceeded until 24 days presumably to achieve ≥4% (*w/v*) volatile acidity. The vinegar was clarified as described for wine.

The semi continuous vinegar fermentation was done with immobilized *A. aceti* cells in an indigenously designed column having a height: diameter of 32: 10 cm. Shredded,



dried and steam sterilized carrier material (sugarcane bagasse, corn cobs), 100 g, was packed in the column and drenched with sterilized water to its maximum water holding capacity till water started oozing out of the carrier material). *A.aceti*, 36 h old 500 ml broth was percolated through the column and left as such for about 12 h for adsorption of cells by adhesion forces between two solid surfaces i.e. carrier material and cells. The wine was made to flow through these columns at 30 and 50 ml/h in repeated cycles of either 4 or 12 h duration each in different experiments until volatile acidity of the product was ≥4% (*w/v*). The fermentation efficiency (FE) was calculated as follows:

$$\%\,\mathrm{FE}\,(\mathrm{Ethanol}) = \frac{\%\,\mathrm{Ethanol\ v/v}}{\%\,\mathrm{sugar} \times 0.64} \times 100$$

$$\%\,\mathrm{FE}\,(\mathrm{Acetic\ acid}) = \frac{\%\,\mathrm{Volatile\ acidity\ w/v}}{\%\,\mathrm{Ethanol\ v/v} \times 1.043} \times 100$$

Analyses TSS was measured as °Brix (°B) using a hand refractometer (Erma, Japan). The total sugars (Lane and Eynon 1923), ethanol (Caputi and Wright 1969), total and volatile acidity as acetic acid (Amerine et al. 1967, AOAC 1980) were estimated. All the experimental treatments were performed in triplicates and the results were subjected to ANOVA.

Results and discussion

Alcoholic fermentation Infusions of 1.0 and 1.5% tea with dissolved sugar (15, 20 and 25%) were prepared where the corresponding estimated sugar contents were 12.8–13.2, 16–16.4 and 22.8–23.1% (Table 1). A gradual fall in TSS during was observed to culminate during 18–24 days of fermentation with final residual sugars levels of 0.6–0.8,

Table 2 Effect of ethanol concentration of tea wine on vinegar fermentation

Tea conc., %	Ethanol conc. of wine, % v/v	Initial acidity, % w/v	Final acidity, % w/v		FE, %
			Total	Volatile	
1.0	6.1	0.5	4.2±0.11	4.0±0.23	63.2
		1.0	4.8±0.11	4.5±0.27	71.4
	8.6	0.5	4.5±0.33	4.3±0.17	48.0
		1.0	4.8±0.33	4.6±0.28	51.6
	12.0	0.5	4.9±0.19	4.5±0.18	36.0
		1.0	5.0±0.28	4.7±0.11	37.5
1.5	6.2	0.5	4.3±0.23	4.2±0.13	65.0
		1.0	4.9±0.31	4.7±0.11	73.0
	8.9	0.5	4.7±0.17	4.5±0.17	48.6
		1.0	5.0±0.17	4.8±0.42	52.0
	12.2	0.5	4.9±0.13	4.7±0.28	37.0
		1.0	5.11±0.33	4.8±0.26	38.0
CD (0.05), (*n*=3)			0.29	0.25	

FE Fermentation efficiency

1.0 and 1.2–1.8% respectively. The increasing initial sugar concentration produced higher levels of ethanol (% *v*/*v*) i.e. 6.1±0.28, 8.6±0.57 and 12.0±0.99 in 1.0% infusion and 6.2±0.37, 8.9±0.71 and 12.2±0.85 in 1.5% tea infusion. The highest FE, 84.3 and 84.8% were however, recorded for 20% sugar in both 1.0 and 1.5% tea infusions respectively. Jiranek et al. (1995) and Kaur and Kocher (2002) also reported 20% sugar as optimum for maximum ethanol production. Variability in alcohol production could be due to metabolic behaviour and adaptability of yeast to different sugar concentrations and fermentation conditions.

Batch fermentation of vinegar Trials conducted on tea wines at different alcohol concentrations and initial acidities reported an upward trend in the production of acidity including volatile acidity (acetic acid) with the rising concentration of ethanol (Table 2). The highest volatile acidities, 4.5±0.18 and 4.7±0.11%, were achieved in 1% tea wine having 12% ethanol and 0.5 and 1.0% initial acidities, respectively. However, the maximum FE, 63.2 and 71.4%, were observed in the 1.0% tea wine with 6.1% ethanol. Similar results were also obtained for vinegar fermentation of 1.5% tea wine i.e. the highest volatile acidity, 4.7±0.28 and 4.8±0.26% from 12.2% ethanol but the maximum FE, 65 and 73% were achieved from 6.2% ethanol. This suggested a negative impact of increased ethanol concentration on acetic acid fermentation (Kim 1999). In contrast to our results, Lu et al. (1999) reported highest acetic acid accumulation at 0.2% initial acidity of the fermentation medium. Kim (1999) reported 7.9–9% ethanol as optimum for its fermentation to vinegar. Sugarcane vinegar having 6% volatile acidity has been produced in almost similar fermentation conditions in our laboratory (Kocher et al. 2003).

Semicontinuous fermentation of vinegar A.aceti cells immobilized on sugarcane bagasse produced the highest total and volatile acidity i.e. 4.7±0.13 and 4.4±0.07% respectively by the semicontinuous fermentation in 36 h duration at a flow rate of 50 ml/h, comprised of 9 flow cycles of 4 h each

Table 3 Effect of carrier materials on acid production by semi continuous vinegar fermentation

Carrier material	Flow rate, ml/h	Wine flow, ml/ cycle	No of cycles	Fermentation period, h	Final acidity, % *w/v*		FE, %
					Total	Volatile	
Sugarcane bagasse	30	120	11	44	4.2±0.17	4.0±0.17	43.2
	50	200	09	36	4.7±0.13	4.4±0.07	47.9
Corn cobs	30	120	12	48	4.3±0.11	4.1±0.14	44.2
	50	200	09	36	4.4±0.23	4.3±0.28	45.9
CD (0.05) (*n*=3)					0.21	0.28	

Conc. of tea and ethanol in wine: 1.5% and 8.9%, v/v, Initial acidity of wine, 1.0% w/v

FE Fermentation efficiency

Table 4 Characteristics of tea vinegar produced by scaling up of semi continuous fermentation

Parameters	Immobilized cells column	
	Sugarcane bagasse	Corn cobs
Total acidity, % w/v	4.6±0.14	4.3±0.11
Volatile acidity, % w/v	4.3±0.06	4.2±0.06
pH	2.4	2.2
Recovery, %	90	85
FE, %	42.7	40.9

Wine ethanol 9.8% v/v, Initial volume 600 ml, Initial acidity 1.0%, pH 3.2, Flow rate 50 ml/h, Duration of one flow cycle 12 h, No. of flow cycles 9, Fermentation period 108 h

FE Fermentation efficiency

and was calculated to be 47.9% FE (Table 3). All other treatments of cell immobilization on bagasse and corn cobs gave statistically similar values of volatile acidity. In scale up of the process, 600 ml 1.5% tea wine at 9.8% alcohol was made to flow through the fermentation column at 50 ml/h. The highest volatile acidity, 4.3±0.06% was achieved in 9 flow cycles of 12 h each in the column of sugarcane bagasse immobilized *A.aceti* cells (Table 4). The process yielded 90% recovery of the product at 42.7% FE. Garg et al. (1995) reported 5.3% acidity and 60% recovery in semicontinuous production of mango vinegar by *A.aceti* cells immobilized on wood shavings. Fregapane et al. (2003) reported 94.2% productivity with inlet flow rate of 950 ml/h. Earlier, in our laboratory, sugarcane vinegar was produced using sugarcane bagasse, corn cobs, wood shavings and calcium alginate immobilized cells in 8 rounds of 10 h each (Kocher et al. 2006).

Conclusion

The present studies successfully brewed vinegar from CTC black tea. Alcoholic and acetous fermentation of upto 24 days each in succession, accomplished production of vinegar of upto 4.8% volatile acids. The semicontinuous fermentation of 600 ml tea wine used immobilized cells of *A. aceti* to achieve 4.3% volatile acidity.

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