

## Original Research Article

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## Fermentative Production of Vinegar from Grapes and Guava Using Adsorbed Cells of *Acetobacter aceti*

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### ABSTRACT

#### Keywords

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Natural vinegar is a food supplement, tonic and nutraceutical produced by twin fermentation of sugar to acetic acid via ethanol. The fermentation of grape and guava juice carried out by *Saccharomyces cerevisiae* MTCC 11815 produced 9.25% (v/v), 9.32% (v/v) of ethanol with a fermentation efficiency of 92.6%, 93.9% in 72 h and 96 h, respectively. The optimized conditions for sugarcane vinegar production were also validated for grape and guava vinegar production up to 5L in PVC column reactors that yield 6.2% (w/v) and 6.1% (w/v) volatile acidity in 8-10 days, respectively. The grape and guava vinegars possessed *in vitro* antioxidant potential with total free radical scavenging activities with EC<sub>50</sub> and AEAC values of 83.4% and 88.9%, 63.6 and 57.0; 0.27 μM and 0.30 μM, respectively. Both the vinegars had a mean sensory score of 7.52±0.75 Grape and 7.60±0.83 Guava in comparison to a commercial brand having 8.48±0.59 score.

### Introduction

India ranks second in fruits and vegetables production in the world, after China with an annual production of 86.602 million metric tonnes of fruits and 169.478 million metric tonnes of vegetables (NHB 2015-16). Among different fruits, grapes and guava have fascinated the local consumers due to their pleasant, sub-acid and aromatic nature. Biochemically, guava is rich in vitamin A (200-400 IU), ascorbic acid (88.2-250.8 mg/100 g), lycopene (45.3 μg/ g FW), total sugars (10-15.3%), reducing sugars (2.05-6.08%), acids (10-15.3%), pectins (0.62%) and phenols (170- 345 GAE/ g FW). At maturity, grape berries possesses water

(74%), sugars (25%, primarily fructose and glucose), organic acids (0.8%, primarily tartaric and malic acids), minerals (0.5%, mainly potassium), phenolics, flavonoids, aromatics and nitrogenous compounds (0.2%) which make them nutritious substrates (Pooja *et al.*, 2016). However, these fruits are marked with a very low shelf-life of about 2-3 days for grapes and 5-7 days for guava at room temperature and thus reflect 10-15% post-harvest losses, which make them ideal candidates for value-addition. Though guava nectars/ juices are available in market, very little work has been carried out towards guava-wine and vinegar production (Kocher

and Pooja, 2011). Natural vinegar is a fermented product of increasing significance by virtue of its widely variable origin and use particularly as a condiment and food preservative. The present industry dealing with the production of natural vinegar still uses the traditional batch fermentation which generally spans 4-5 weeks (Lea 1989; Fregapane *et al.*, 2003; Sossou *et al.*, 2009). Batch scale technologies for sugarcane and grape vinegar fermentation at 50L scale were earlier developed in our laboratory that took 25-28 days for producing vinegar (Kocher *et al.*, 2014). Since immobilized cells technique is known to enhance fermentation (Kocher *et al.*, 2006), a semi-continuous sugarcane vinegar production technology using wood shaving adsorbed cells has also been developed with a reduced fermentation time without compromising vinegar quality (Kumar and Kocher, 2016). In the present study, the latter has been standardized and validated for two types of vinegar production viz. grapes and guava.

### **Materials and Methods**

Grape juice and guava juice had a brix of  $17.0 \pm 2$  and  $5.0 \pm 1.5$  °B, respectively and a pH of  $4.5 \pm 0.2$  were observed for both fruits. Brix-acid ratio of guava juice was adjusted in the desirable range by using sugar (raising Brix to  $17.0$ °B) and citric acid, while the same was already found in the desirable range in case of grapes. The cultures used in the study viz; *Saccharomyces cerevisiae* MTCC 11815 and *Acetobacter aceti* AC1 were local isolates of our laboratory.

### **Ethanol fermentation**

Ethanol fermentation of grape and guava juice (50L) were performed by inoculating freshly prepared 24 h old inoculum of *S. cerevisiae* MTCC 11815 (in jaggary solution @ 150 g/l) @ 6% (v/v) and 9% (v/v),

respectively followed by incubation at  $28 \pm 2$ °C (Pooja *et al.*, 2014; Joshi, 2010) till the bubbling ceased and 'lees' settled at bottom of the container. The final ethanol concentration was analysed by the dichromate oxidation method (Caputi and Wright, 1969).

### **Acetic acid fermentation**

The acetic acid fermentation of grape and guava ethanol was carried out in 7L plastic column fermenters. The conditions for adsorption of *A. aceti* cells and packing length were earlier optimized for sugarcane vinegar production in indigenously prepared plastic columns having capacity of 1L and 7L with working volumes of 800ml and 5L, respectively (Kumar and Kocher, 2016). These standardized conditions were validated for grape and guava vinegar production in 5L scale. Each column was packed with *A. aceti* cells adsorbed wood shavings and charged with grape and guava alcohol mixed with mother vinegar in a ratio of 3:2, so as to have an initial acidity of 2% (w/v). The fermenters were incubated at  $28 \pm 2$ °C and used to measure volatile acidity (AOAC 1980) and residual alcohol as discussed earlier, The results of fermentation were analysed statistically using CPCS1.

### **In vitro antioxidant potential**

The *in vitro* antioxidant potential of fermented vinegar was estimated as total free radical scavenging activity by DPPH method (Sanchez-Moreno *et al.*, 1999). The EC<sub>50</sub> and AEAC values of fermented vinegar were also calculated by the method of Shimamura *et al.*, (2014).

### **Storage and sensory analysis**

The grape and guava vinegars produced were stored at 4°C, for 3-4 days, and the settled bacterial cells and sediment were separated.

The partially clarified vinegars were bottled, pasteurized (using a water bath at 65°C for 30 min) and stored at room temperature. The sensory analysis of at least 3-months-old vinegars was performed by 10 judges at a modified 10 point Hedonic scale (Amerine and Roessler, 1976), which included five parameters viz; appearance, colour, astringency, sourness, bouquet and compared with a commercial brand.

## Results and Discussion

### Ethanol fermentation

The physicochemical analysis of grape juice revealed a TSS content of 17.0°B with total and reducing sugars of 16.2 and 15.52% (w/v), respectively. The ethanolic fermentation of grape juice using *S. cerevisiae* MTCC 11815 (6% w/v) was ceased in 72 h producing an ethanol of 9.25% (v/v) with a fermentation efficiency of 85.0% (Table 1) under conditions optimized earlier (Pooja, 2016). In literature, Kocher *et al.*, (2009) recorded 11.04% (v/v) grape ethanol production from 20°B with fermentation efficiency of 90%. Yan *et al.*, (2009) reported ethanol production of 143.8 g/l from grapes.

The fermentation of guava juice (adjusted to 17.0°B) also carried out using *S. cerevisiae* MTCC 11815 (9% w/v) was ceased in 96 h producing an ethanol of 9.32% (v/v) from reducing sugars (15.85% w/v) with a fermentation efficiency of 85.6% (Table 1).

Pooja and Kocher (2014) optimized the guava ethanol production conditions leading to production of guava ethanol in the range of 12.0-13.0% (v/v) in 6 days with fermentation efficiency of 81%. Srivastava *et al.*, (1997) reported that 10% inoculum size added in Guava pulp led to the production of 5.8% ethanol (w/v) by *S. cerevisiae*. Sveda and Rodrigues (2011) optimized 22°B and 25°C

and 0.06% Diammonium phosphate (DAP) concentration for guava must fermentation.

### Acetic acid fermentation by adsorbed cells

In our earlier study, a half length packed PVC column with the *Melona grandis* (15mm) wood shavings adsorbed cells (in the ratio of 2:1 with *A. aceti* for 15h with 0.2% DAHP supplementation at 28°C) produced sugarcane vinegar in 6 days from a initial acidity of 2% (w/v) (Kumar and Kocher, 2016). These optimized conditions on sugarcane vinegar production were validated for grape and guava vinegar production at 7L scale. The results presented in Table 2 revealed production of grape and guava vinegar with high acidity of 6.2% (w/v) and 6.1% (w/v), respectively in 8-10 days which is more than that of sugarcane vinegar as well as the limits prescribed by FSSAI (Gaur, 2011).

Earlier, De Ory *et al.*, (2004) reported vinegar production in 225L pilot plant producing high quality vinegar with 100% yield. Similarly, Krusong and Vichitroka (2011) reported corn vinegar production in a recycling 10L semi-continuous fermentation system producing high acidity (6.8-7.2 % w/v) vinegar in 4-5 days.

### In vitro antioxidant potential

#### Estimation of free radical scavenging activity for DPPH

The DPPH scavenging activity (Fig. 1) of grape and guava vinegar was tested that revealed EC<sub>50</sub> values of 63.6 and 57.0 µM with AEAC values of 0.27 and 0.30 µM, respectively (Table 3). Further, EC<sub>50</sub> of ascorbic acid taken as positive control was 17.2 µM.

Earlier, grape juice has been shown to possess DPPH activity in the range of 8.23 ± 0.17,

2.51 ± 0.03 and 8.24 ± 0.19 mM in homemade, commercial and organic juice, respectively (Burin *et al.*, 2010). Wine vinegar is also reported to contain significantly higher total polyphenol content and hence possess greater antioxidant capacity compared to distilled vinegar (Pinisodom *et al.*, 2010). The ORAC-FL values varied from 14.6 to 25.0 µmol of trolox

equivalents/ml for red grape juices, from 3.5 to 11.1 µmol of trolox equivalents/ml for white grape juices, and from 4.5 to 11.5 µmol of trolox equivalents/ml for wine vinegars (Alberto *et al.*, 2005). Guava wines from Hisar Safeda and Hisar surkha were found to have antioxidant activity of 26.2 and 26.4%, respectively (Sharma, 2015).

**Table.1** Ethanolic fermentation of Grape and Guava juice by *S. cerevisiae* MTCC 11815

| Grape <sup>a</sup>      |            |              |                 |          |                 |
|-------------------------|------------|--------------|-----------------|----------|-----------------|
| Fermentation Period (h) | TSS (Brix) | Total Sugars | Reducing Sugars | pH       | Ethanol (% v/v) |
| 0                       | 17±0.20    | 16.1±0.13    | 15.6±0.12       | 4.8±0.05 | 0.0             |
| 24                      | 11±0.15    | 9.9±0.14     | 9.3±0.07        | 4.6±0.1  | 3.5±0.05        |
| 48                      | 2.5±0.10   | 1.3±0.08     | 0.7±0.05        | 4.4±0.05 | 8.5±0.07        |
| 72                      | 0.0        | 0.4±0.07     | 0.0±0.06        | 4.1±0.05 | 9.25±0.05       |
| FE (%)                  | 92.6       |              |                 |          |                 |
| Guava <sup>b</sup>      |            |              |                 |          |                 |
| 0                       | 17±0.15    | 16.4±0.11    | 15.5±0.7        | 5.5±0.05 | 0.0             |
| 24                      | 13±0.15    | 12.2±0.08    | 11.6±0.10       | 5.2±0.04 | 1.9±0.1         |
| 48                      | 7.5±0.20   | 6.4±0.05     | 5.3±0.08        | 4.8±0.05 | 5.4±0.07        |
| 72                      | 2.5±0.10   | 1.4±0.04     | 0.7±0.04        | 4.6±0.1  | 8.3±0.05        |
| 96                      | 0.0        | 0.7±0.03     | 0.0±0.03        | 4.4±0.05 | 9.32±0.05       |
| FE (%)                  | 93.9       |              |                 |          |                 |

**Cultural conditions:**

Scale of fermentation : 50 L      Temperature<sup>a</sup>: 28±2°C      Temperature<sup>b</sup>: 25±2°C  
 Inoculum<sup>a</sup> : 6% (v/v)      Inoculum<sup>b</sup> : 9% (v/v)

**Calculations:**

$$\text{Fermentation Efficiency (FE)} = \frac{\text{Actual ethanol produced}}{\text{Theoretical ethanol produced}} \times 100$$

Theoretical Ethanol% (v/v) = Sugar utilized × 0.64

Sugar Utilized (on brix basis) = Available sugar - Sugar present after fermentation

**Table.2** Semi-continuous fermentation of grape and guava ethanol in packed bed fermenters at 5L scale

| Fermentation cycles         | Grape <sup>a</sup> |          |         | Guava <sup>b</sup> |          |          |
|-----------------------------|--------------------|----------|---------|--------------------|----------|----------|
|                             | Initial            | Final    | Days    | Initial            | Final    | Days     |
| 1                           | 2.0                | 6.2      | 11      | 2.0                | 6.0      | 13       |
| 2                           | 2.3                | 6.1      | 8       | 2.8                | 5.9      | 9        |
| 3                           | 2.1                | 6.6      | 7       | 2.1                | 6.0      | 9        |
| 4                           | 2.3                | 7.0      | 7       | 2.2                | 6.5      | 10       |
| 5                           | 2.1                | 5.9      | 9       | 2.1                | 5.2      | 7        |
| 6                           | 2.6                | 5.5      | 8       | 2.2                | 5.6      | 8        |
| 7                           | 2.6                | 5.8      | 9       | 2.0                | 6.7      | 9        |
| 8                           | 2.2                | 6.3      | 8       | 2.0                | 7.1      | 8        |
| 9                           | 2.1                | 6.1      | 7       | 2.5                | 5.8      | 9        |
| 10                          | 2.4                | 6.2      | 7       | 2.4                | 6.5      | 8        |
| Mean±S.D.                   | 2.3±0.21           | 6.2±0.41 | 8.1±1.3 | 2.2±0.26           | 6.1±0.56 | 9.0±1.63 |
| Fermentation efficiency (%) | 79.6               |          |         | 78.3               |          |          |
| Yield (g/g)                 | 0.78               |          |         | 0.76               |          |          |
| CD <sub>5%</sub>            | 0.083              |          |         |                    |          |          |

**Fermentation conditions:**

|                               |                  |                               |                  |
|-------------------------------|------------------|-------------------------------|------------------|
| Temperature <sup>a</sup>      | : 28±2°C         | Temperature <sup>b</sup>      | : 28±2°C         |
| Initial alcohol <sup>a</sup>  | : 7.0% (v/v)     | Initial alcohol <sup>b</sup>  | : 7.5% (v/v)     |
| Residual alcohol <sup>a</sup> | : 0.5±0.2% (v/v) | Residual alcohol <sup>b</sup> | : 0.7±0.2% (v/v) |

**Table.3** DPPH scavenging activity in respect of different concentrations of Grape and Guava vinegar

|                     | Concentration (µM) | % DPPH scavenging activity | EC <sub>50</sub> Value (µM) | AEAC (µM) |
|---------------------|--------------------|----------------------------|-----------------------------|-----------|
| Ascorbic acid (1mM) | 20                 | 55.1                       | 17.2                        | 1.0       |
|                     | 40                 | 94.7                       |                             |           |
|                     | 60                 | 95.6                       |                             |           |
|                     | 80                 | 96.3                       |                             |           |
|                     | 100                | 96.9                       |                             |           |
| Grape vinegar       | 10                 | 5.78                       | 63.6                        | 0.27      |
|                     | 50                 | 39.4                       |                             |           |
|                     | 100                | 83.4                       |                             |           |
| Guava vinegar       | 10                 | 7.27                       | 57.0                        | 0.30      |
|                     | 50                 | 41.5                       |                             |           |
|                     | 100                | 88.9                       |                             |           |

$$AEAC = \frac{EC_{50} \text{ of ascorbic acid } (\mu\text{M})}{EC_{50} \text{ of samples } (\mu\text{M})}$$

**Table.4** Sensory evaluation of vinegar produced by semi-continuous fermentation and commercial vinegar

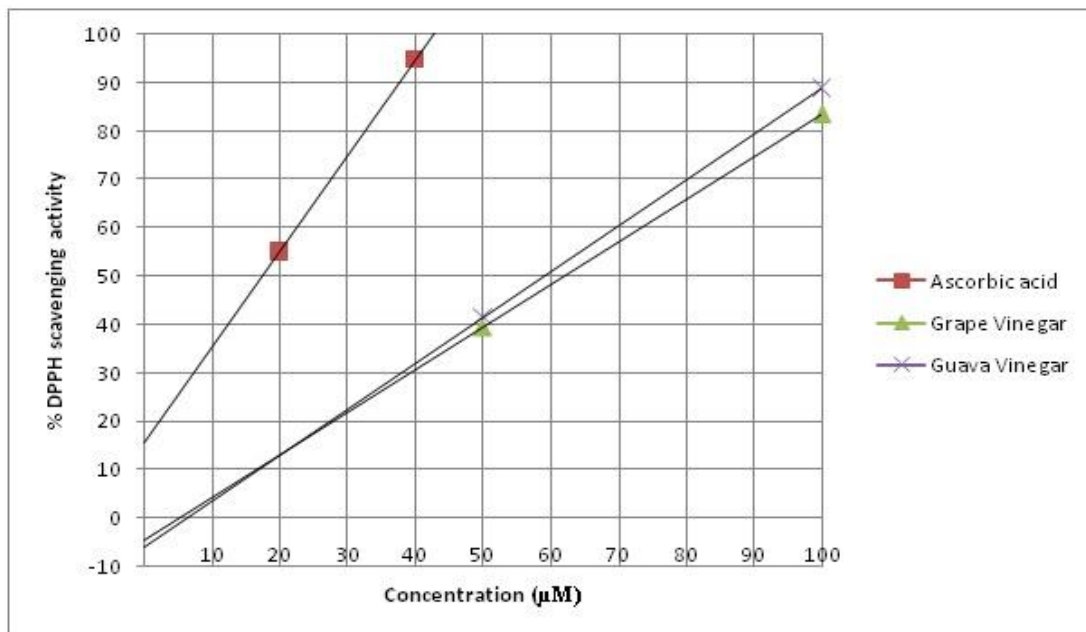
| Sensory analysis   | Maximum Points | Vinegar sensory score* |           |           |
|--------------------|----------------|------------------------|-----------|-----------|
|                    |                | Commercial             | Grape     | Guava     |
| <b>Appearance</b>  | 2              | 1.74±0.06              | 1.81±0.02 | 1.73±0.08 |
| <b>Colour</b>      | 2              | 1.58±0.14              | 1.78±0.07 | 1.74±0.07 |
| <b>Astringency</b> | 2              | 1.79±0.15              | 1.25±0.22 | 1.21±0.26 |
| <b>Sourness</b>    | 2              | 1.68±0.06              | 1.48±0.27 | 1.55±0.19 |
| <b>Bouquet</b>     | 2              | 1.69±0.18              | 1.20±0.17 | 1.37±0.23 |
| <b>Total</b>       | 10             | 8.48±0.59              | 7.52±0.75 | 7.60±0.83 |

\* The above scoring is mean (±) standard deviation of evaluation by 10 penalists

\* Sensory quality:

9-10: Outstanding vinegar, 7-8.99: Standard vinegar, 5-6.99: Commercial vinegar, 3-4.99: Below commercial vinegar acceptability, 1-2.99: Spoiled vinegar

**Fig.1** Percent DPPH scavenging activity of grape and guava vinegar with ascorbic acid as positive control for calculating EC50 values ( $y=Ax+B$ )



### Storage and sensory analysis

The sensory analysis of aged vinegars (at least 3 months old) was carried out at 10 point hedonic scale to find out its acceptability among the tasters. The vinegar produced by semi-continuous method over the 10 cycles was found to be consistent in terms of sensory

attributes. It was found that grape and guava vinegars were acceptable with a mean score of 7.52±0.75 and 7.60±0.83, whereas mean score of vinegar produced commercially was 8.48±0.59, respectively (Table 4). The results showed that both the vinegars produced by semi-continuous method as of standard quality and there is not much difference in the

sensory qualities of vinegar produced commercially. In literature, Kumar and Kocher (2016) analysed the characteristics of sugarcane vinegar produced by semi-continuous fermentation on hedonic scale and categorised it of standard quality. Sharma (2015) studied the sensory characteristics of guava vinegar in terms of color, aroma, taste and overall acceptability and rated the guava vinegar in superior quality range with a score of 8.0 out of 10.0.

In conclusion, in the present study, semi-continuous fermentation vinegar production in respect of guava and grapes was successful accomplished at 5L scale in indigenous PVC column reactors produces 6.2% (w/v) and 6.1% (w/v) volatile acidity in 8-10 days, respectively. The grape and guava vinegar possessed *in vitro* antioxidant potential with total free radical scavenging activity of 83.4% and 88.9%, respectively, thus revealing potential commercial applications of the developed economical technology.

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