Preparation and quality evaluation of vinegar prepared by Acetobacter aceti

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ABSTRACT

In present study vinegar was prepared from alcohol by using Acetobacter aceti. For this purpose culture was used at the rate of 30%, 40%, 50% and 60% with a viable count of 10^6 cfu/mL. The vinegar thus prepared was analyzed for different attributes like flavour, taste, overall acceptability, pH, acidity, total soluble solids, volatile acids and nonvolatile acids after 20 days of fermentation in orbital incubator at 30±2°C. On the basis of these results it was concluded that culture dosage significantly affected the physico-chemical and sensory quality of the vinegar.

Key words: Acetobacter, Vinegar, Vinegar analysis

INTRODUCTION

Vinegar is the product of a mixed fermentation of yeast followed by acetic fermentation. Acetic acid produced by the fermentation of alcohol (ethanol) gives the characteristic flavor and aroma to vinegar. It can be made from almost any fermentable carbohydrate source, for example fruits, vegetables, syrups and wine. The basic requirement for vinegar production is a raw material that can be subjected to alcoholic fermentation. Apples, pears, grapes, honey, syrups, cereals, hydrolyzed starches and wine are all ideal substrates for the production of vinegar. The raw material used as substrate should be well matured, clean and in good healthy conditions (Tortora et al. 1995).

Strains of acetic acid forming bacteria (Acetobacter) and oxygen to enable the oxidation of alcohol are necessary in an alcoholic substrate. However, this process is very slow and vinegars produced by this method tend to be of inferior quality. Controlled fermentation conditions produce a more acceptable product by using a wide range of raw materials for vinegar (Battcock and Ali, 1998).

In Pakistan, although synthetic vinegar is mostly consumed yet fermented vinegar is also available. The culture used for the production of fermented vinegar is mostly contaminated with other unnecessary micro-organisms at industrial level. Hence it is the need of the time to develop pure vinegar cultures to improve the quality of fermented vinegar so that the use of synthetic vinegar may be avoided as it is prohibited in most European and overseas countries (Rehman and Reed, 1983).

Furthermore the Food and Agriculture Organization of the United Nations (FAO) has established that vinegar is a liquid allowed for human consumption of two consecutive fermentations, first an alcoholic fermentation that transforms the sugar into ethanol and then acetic fermentation that converts ethanol to acetic acid, that is the main product of vinegar (Parrondo et al. 2003). Keeping in view the study was conducted for isolation of vinegar culture which subsequently used for vinegar production.

MATERIALS AND METHODS

Culture collection

Vinegar culture Acetobacter aceti after isolation, identification and characterization was produced in Food Microbiology and Biotechnology Laboratory, Institute of Food Science and Technology, University of Agriculture, Faisalabad by following the criteria suggested by Holt et al. (1994).

Vinegar preparation

Raw material

Alcohol: Alcohol was obtained from Mitchell’s Fruit Farms Ltd. Okara.

Vinegar culture: A pure culture of Acetobacter aceti isolated with a viable count of 10^6 cfu/ml (colony forming unit/ ml) was used at different levels as given below. Viability was measured by the methods recommended by Awan and Rahman 2002 and Cappuccino and Sherman 1996.
Different levels of culture used for preparation of vinegar

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Culture percentage</th>
<th>Alcohol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>T2</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>T3</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>T4</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>

**Vinegar making**

Acetobacter aceti was added in to alcohol (sterilized by filtration) in 250 ml conical flask and fermentation was carried out in an orbital shaker at 30± 2°C with 250 rpm (Parrondo et al. 2003) for 20 days. Samples were examined for aroma and acidity on daily basis until specific aroma and acidity for vinegar preparation was achieved.

**Pasteurization**

When the required criterion of acidity and aroma was achieved, vinegar samples were pasteurized in water bath at temperature of 60°C for 90 seconds in sterilized bottles to check any further fermentation as suggested by Riaz and Ahmad 1996.

**Cooling**

Vinegar samples were cooled down to 25°C for evaluation.

**Vinegar evaluation**

**Quality tests for vinegar**

pH, acidity, soluble solids (Brix), total acids, non volatile acids and volatile acids were determined to evaluate the quality of vinegar according to the procedures explained in AOAC (1990).

**Sensory evaluation of vinegar**

In order to check the overall acceptability of vinegar, the samples were submitted for sensory evaluation. Vinegar was evaluated for its color, flavor, taste and overall acceptability by a panel of trained judges using the 9-point Hedonic scale as suggested by Larmond (1977).

**Statistical analysis**

Data obtained was statistically analyzed to see the effect of different doses of culture on the quality of vinegar by the method as recommended by Steel et al., 1997.

**RESULTS AND DISCUSSION**

**Physico-chemical analysis of vinegar**

The results obtained are discussed as under.

**pH**

The results for pH given in Table 1 indicate that pH of vinegar produced was significantly affected by the culture dosage as the highest pH value was observed in case of T1 (30 percent) with the value 2.60, whereas this figure was reduced to lowest figure 2.42 when T4 was analyzed. It is obvious from the results that the pH value gradually decreased from T1 to T4 (2.60, 2.50, 2.49 and 2.42) as dose was 30 percent, 40 percent, 50 percent and 60 percent respectively. Furthermore as analysis of variance given in Table 2 indicates that there was a significant effect of treatments on the pH of the end product however, the differences in pH of T2 and T3 were non-significant. The significant results obtained are similar to the recommendations of Rehm and Reed 1983 who recommended that vinegar has a pH range of 2.35 to 2.45.

**Acidity (total acids)**

The results for acidity as recorded are expressed in Table 1. It was found that the treatment T4 gave significantly the highest mean value (5.58 percent) for acidity whereas the lowest mean value (2.60 percent) was seen in the treatment T1. The mean acidity values measured for T3 and T4 were 3.96 percent and 4.51 percent respectively. Moreover statistical results given in Table 2 indicate that the effect of different culture percentages on the quality of vinegar was highly significant. Although T4 was found to be the highest acidity value but the range for this parameter in the present study was 2.6-5.58 percent. The upper values of acidity in T2 and T4 fell within the range as concluded by Rehm and Reed 1983 who reported that usually vinegar has 4-7 percent acidity. T2 (3.9 percent) was also nearest to the lowest acidity value quoted by them.

**Total soluble solids**

Results obtained for total soluble solids indicated (Table 1) that significantly the highest total soluble solids were found in T1 with a mean value of 12.16 percent. Similarly the lowest total soluble contents were calculated in T4 (10.16 percent). Non-significant in results for total soluble solids in T2 and T3 as well as among T2 and T3 but significant difference was obtained in T1 and T4. Furthermore results for analysis of variance given in Table 2 exhibited that the effect of different percentages of culture was highly significant.
on total soluble solids of the end product. It is due to the reasons that more the number of bacteria more will be the utilization of sugars and ultimately there will be reduction in total soluble solids in the end product.

Nonvolatile acids

Table 1 showed that significantly the highest nonvolatile acids were determined in T<sub>4</sub> with mean value 0.38 percent and significantly the lowest percentage of nonvolatile acids was measured in T<sub>1</sub>, T<sub>3</sub> and T<sub>4</sub> each having a non-significant mean value of 0.35 percent. However, the effect of culture percentage was clearly found to be highly significant on the concentration of nonvolatile acids in vinegar as shown in Table 2.

Table 1. Effect of culture dosage on chemical composition of vinegar

<table>
<thead>
<tr>
<th>Culture (%)</th>
<th>pH</th>
<th>Acidity (% acetic acid)</th>
<th>Soluble solids (%age)</th>
<th>Nonvolatile acids (%age)</th>
<th>Volatile acids (%age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt; (30)</td>
<td>2.60a</td>
<td>2.60d</td>
<td>12.16a</td>
<td>0.35b</td>
<td>2.25d</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt; (40)</td>
<td>2.50b</td>
<td>3.96c</td>
<td>11.66ab</td>
<td>0.35b</td>
<td>3.61c</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; (50)</td>
<td>2.49b</td>
<td>4.51b</td>
<td>11.16b</td>
<td>0.35b</td>
<td>4.16b</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (60)</td>
<td>2.42c</td>
<td>5.58d</td>
<td>10.16c</td>
<td>0.38a</td>
<td>5.65a</td>
</tr>
</tbody>
</table>

Results are given as means of three observations
Values with same letters are non-significant with one another

Table 2: Analysis of variance for chemical composition of vinegar

<table>
<thead>
<tr>
<th>Sources</th>
<th>DF</th>
<th>pH</th>
<th>Acidity (% acetic acid)</th>
<th>Soluble solids (%age)</th>
<th>Nonvolatile acids (%age)</th>
<th>Volatile acids (%age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>0.015933**</td>
<td>4.6239**</td>
<td>2.187**</td>
<td>0.000608**</td>
<td>5.9430**</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.000233</td>
<td>0.0106</td>
<td>0.208</td>
<td>0.000133</td>
<td>0.0118</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Highly significant (p > 0.01)

Volatile acids

Results given in Table 1 indicate that all the treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were significantly different from each other. The treatment T<sub>4</sub> was significantly at the top with a mean value of 5.65 percent followed by T<sub>4</sub> while treatment T<sub>1</sub> was significantly at the bottom with volatile acid contents of 2.25 percent. The mean values for volatile acid contents in T<sub>2</sub> and T<sub>3</sub> were 3.61 percent and 4.16 percent respectively. Analysis of variance given in Table 2 revealed that the effect of culture percentage was highly significant on the volatile acid contents on vinegar and T<sub>3</sub> (7.00) were non-significantly with each other. Analysis of variance showed that there was a highly significant effect of culture percentage on color of the vinegar as indicated in Table 4.

Sensory evaluation of vinegar

The results regarding sensory evaluation are presented in Table 3 along with statistical data in Table 4.

Color

Color plays an important role in the visual evaluation and aesthetic appeal of a food product. Different culture percentages had different effect on the color of the vinegar. Significantly the highest scores were given to the color of the vinegar prepared by T<sub>2</sub> (8.20) while treatment T<sub>1</sub> obtained lowest score (6.70). It was further observed from the Table 3 that treatment T<sub>4</sub> was highly significantly different from the rest of the treatments whereas treatments T<sub>1</sub> (6.70), T<sub>2</sub> (6.90)
Table 4. Analysis of variance for sensory attributes of vinegar

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Taste</td>
</tr>
<tr>
<td>Treatment</td>
<td>3</td>
<td>2.3000**</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>0.0500</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

** Highly significant (p > 0.01)

Flavor

Flavor also plays a key role in the sensory evaluation of a food product. The results expressed in the Table 3 showed that significantly the highest scores for flavour were given to the vinegar prepared from T4 (7.70) followed by T3 (6.80) and significantly the lowest scores were given to the vinegar of T1 (5.70) with second lowest figure T2 (6.20) which were non-significant with each other. Typical flavour of the vinegar is due to Acetobacter acetii, which produce acetic acid and more the number of bacteria fermentation becomes faster, more acetic acid is produced and hence best results for flavour are achieved. More acetic acid formation occurred with higher doses of vinegar culture and hence maximum flavour score was obtained.

Taste

For taste of vinegar, it was used in chicken soup and then soup prepared by using vinegar obtained from different treatments was subjected for evaluation. It was found from the Table 3 that vinegar prepared from treatment T4 was significantly at the top with mean scores of 8.20 followed by T3 with mean scores of 6.90 and it was highly accepted by the judges. Significantly the lowest scores were given to vinegar of T1 and T2 with mean values of 5.80 and 6.50 respectively and these treatments were non-significant with other. ANOVA results shown in Table 4 indicate that the taste of the vinegar was highly significantly affected due to different concentrations of the cultures used for acetic acid fermentation.

Overall acceptability

Overall acceptability is one of the important and basic features for the acceptance or rejection of a food product. It is clear from the Table 3 that vinegar prepared from treatment T4 with average score 8.20 was highly accepted by the judges and treatment T1 with mean scores of 5.80 was found significantly much poor. Further more treatment T2 (6.80) and treatment T3 (6.90) were non-significantly different from each other. Results for analysis of variance are expressed in Table 4 indicated that there was highly significant effect of treatments on overall acceptability of the vinegar prepared from different treatments.

Results are similar to the recommendations of Rehm and Reed 1983 who stated that the acetic acid is the typical constituent of vinegar and that more the number of viable count (culture percentage) more will be acetic acid production. If there is more production of acetic acid then ultimately sensory attributes and chemical composition will be affected.

LITERATURE CITED


