Studies on production of alcoholic beverages from some tropical fruits

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Abstract The varieties of alcoholic beverages from watermelon; watermelon-banana and watermelon-pineapple mixtures were produced by using monoculture and mixed culture fermentation techniques. Three yeast species, namely, *Saccharomyces cerevisiae*, *Kleochera apicalata*, *Torulaspora delbruckii* and four bacterial species *Leuconostoc oenos*, *Lactobacillus Sp*, *Micrococcus luteus* and *Streptococcus lactis* were identified during the study. The daily succession of these organisms in the various fermenting samples differed in cell mass and occurrence due to their different growth conditions and factors present. A higher bacterial load (3.9 ± 0.2–4.4 ± 0.3) log (cfu) ml–1 than yeast (2.8 ± 0.0–4.6 ± 0.4) log (cfu) ml–1 counts was observed in the mixed culture fermentation, while in the monoculture fermentation, a higher yeast load (4.3 ± 0.3–4.7 ± 0.2) log (cfu) ml–1 than bacterial loads (2.7 ± 0.1–4.1 ± 0.3) log (cfu) ml–1 counts was recovered. The results obtained from the present study indicated that monoculture-fermented beverages were of better quality as compared to the mixed culture fermented ones. The monoculture-fermented beverage from watermelon-pineapple mixture was ranked as the best alcoholic beverage based on sensory evaluation score.

Keywords Beverages · Fermentation · Microbiological quality · Tropical fruits

Introduction

The wine is any alcoholic beverage produced from juices of variety of fruits by fermentative action of microorganisms, either spontaneously or seeding with a particular strain mainly of yeast species, to adopt a particular quality of wine. Principally, distinctive flavours of wine originate from raw materials during alcoholic and malolactic fermentation [1]. All over the world, different raw materials are used for the production of alcoholic beverages traditionally. The forms of alcoholic beverage consumed in various regions of the world vary considerably in accordance to location and ingredients [2].

There has been some controversy over the relative merits of spontaneous fermentations with natural flora of the ‘must’ and fermentation carried out with selected yeast strains. While it was found that spontaneous fermentation produced a better rounded and more complex aromatic quality [3], a subsequent study concluded it a significant preference for wine produced with selected yeast [4].

Seeding of the fermentation is undertaken with the assumption and expectation that the inoculated strain will out-compete and dominate over indigenous strains of *Saccharomyces cerevisiae* and the non-saccharomyces yeasts. Although there is high probability that inoculated *S. cerevisiae* will dominate the fermentation, seeding will not necessarily guarantee the dominance of any particular strain or its exclusive contribution in the fermentation [5, 6].

This study is expected to provide information on microbial population succession in alcoholic beverage produced...
from some tropical fruits through mixed and monoculture fermentation, and the effect of fermentation strategy and fruit mixture on the beverage quality.

**Materials and methods**

Watermelon (*Citrullus lunatus* Thumb) banana (*Musa sapientum*) and pineapple (*Ananas comosus*) were procured from local farms in the close vicinity to Moradabad, U.P., India. All containers and appliances used in the study were properly sterilized and the fruits were processed under aseptic conditions.

The first treatment set up involved a homogenate of 8000×g of watermelon. The 2nd treatment contained 8000×g each of homogenized watermelon and banana while the 3rd treatment was a homogenate of 8000×g each of watermelon and pineapple. However, each of the treatments was replicated for (monoculture and mixed culture) fermentations.

The fermentation was carried out at room temperature of 28 ± 2°C. In the mixed culture fermentation, indigenous microflora of the fruits were allowed for the fermentation while in the monoculture, the substrates were seeded with *Saccharomyces cerevisiae* of quantity 8.62 log cfu ml–1 to overgrow the indigenous microflora of the fruits. All the treatments were allowed to ferment for 7 days.

At every 24 h, the samples were aseptically withdrawn from the Fermenters, serially diluted and 1ml each pure laced in triplicates on nutrient agar (NA) and incubated at 30°C for 24 h (bacterial growth) and potato dextrose agar (PDA) incubated at 28°C for 72 h (molds and yeast growth), in accordance to a standardized protocol [7]. Resultant colonies were enumerated with the aid of Gallenkamp colony counter, purified by streaking technique on freshly prepared NA and PDA; characterized and identified by a standard protocol [8]. Yeasts were identified by using Kreger [9].

The filtered beverage samples were tested for sensory evaluation using the multiple comparison tests [10]. The sensory parameters evaluated were taste, colour, aroma and overall acceptability. The filtered beverage samples were served chilled in white glass cups in an open space under bright daylight. With a 10-member panel of regular local beverage consumers, three glass cups each from each replicate were served. The parameters were rated on a 9-point hedonic scale. The Dunnett test was applied to determine significant results. The ratings were described as dislike extremely (1), dislike very much (2), no preference (5), like slightly (6), like moderately (7), like very much (8) and like extremely (9).

The data obtained were analyzed using the analysis of variance (ANOVA) to determine differences [11, 12] and Duncan’s Multiple Range Test (DMRT) to separate the means [13].

**Results**

Seven different microorganisms were identified. Four were bacterial species including *Micrococcus luteus*, *Leuconostoc oenos*, *Lactobacillus* sp and *Streptococcus lactis* while three were yeast species, namely, *Kleoxekera apiculata*, *Torulospora delbruckii* and *Saccharomyces cerevisiae*. Among these organisms, only *Saccharomyces cerevisiae* persisted throughout the period of fermentation in both the monoculture and mixed cultures. *Leuconostoc oenos* and *Lactobacillus* sp were not identified in the fermenting musts at the early stages of fermentation but were prominent toward the end of the fermentation duration. On the other hand, *K. apiculata* and *T. delbruckii* were present in the first two days of fermentation and could not be isolated from the must thereafter.

*S. cerevisiae* counts in the fermenting musts showed an increasing trend during the course of fermentation. Though substantial yeast counts were recorded in the mixed culture fermentation, it was found higher in the monoculture fermentation.

Generally, the mixed culture fermented samples had more bacterial population (Table 1) as compared to yeast (Table 2). In the watermelon fermenting medium, bacterial counts

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**Table 1** Total bacterial counts (log (cfu) ml–1 ± S.D) during the course of mixed culture fermentation. Values are means ± S.D of three sets of experiments with triplicate in each set.

<table>
<thead>
<tr>
<th>Day</th>
<th>Watermelon</th>
<th>Watermelon+Banana</th>
<th>Watermelon+Pineapple</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.3 ± 0.1</td>
<td>4.3 ± 0.3</td>
<td>3.9 ± 0.2</td>
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<td>1</td>
<td>4.3 ± 0.1</td>
<td>4.3 ± 0.3</td>
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<td>2</td>
<td>4.3 ± 0.2</td>
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<td>3</td>
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<td>4</td>
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<td>5</td>
<td>4.4 ± 0.3</td>
<td>4.4 ± 0.3</td>
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