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Short communication

Yeast and lactic acid flora of *tej*, an indigenous Ethiopian honey wine: Variations within and between production units

Bekele Bahiru[a], Tetemke Mehari[b], Mogessie Ashenafi[c,*]

[a]*Department of Biology, Faculty of Science, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia*
[b]*Department of Chemistry, Faculty of Science, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia*
[c]*Institute of Pathobiology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia*

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Abstract

A total of 200 samples of *tej*, an indigenous Ethiopian honey wine, were collected from ten production units at different production times. The samples were analysed for their microbial flora. Mean counts of aerobic mesophilic bacteria and aerobic spores for the different production units were <3 log cfu/ml. Coliforms and other members of Enterobacteriaceae were below detectable levels basically due to the low pH of the samples (<4.0). Yeasts were among the dominant micro-organisms in all samples with mean counts of 6 log cfu/ml for all production units. Over 25% of the yeast isolates belonged to *Saccharomyces cerevisiae* followed by *Kluyvermyces bulgaricus* (16%), *Debaromyces phaffi* (14%) and *K. veronae* (10%). Yeast counts showed significant variation within samples of a production unit (CV > 10%) and difference in counts among all samples was also significant ($P<0.01$). The lactic acid bacteria had counts of 6 log cfu/ml with a significant variation within samples of a production unit (CV > 10%) or among all samples ($P<0.01$). In most production units, the heterolactics had higher counts than the homolactics. The lactic flora consisted of *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Pediococcus* species. The lactobacilli were, however, the most frequently encountered groups. In most of the samples, the lactic flora was dominated by two (49.5%) or three (46%) groups of lactic acid bacteria.

Keywords: *Tej*; Indigenous fermentation; Micro-organisms; Variability

1. Introduction

In many African countries, consumption of yeast and lactic acid fermented products is common. Indigenous fermented alcoholic beverages from different parts of Africa such as Egyptian bouza, Tanzanian Wanzuki, gongo, tembo-mnazi and gara, Nigerian palm-wine, Kenyan muratina and uragua, and South African kaffir beer are described in Steinkraus (1983). Popular among the indigenous Ethiopian fermented beverages are tej, tella, borde and shamita (Ashenafi, 2002).

Honey wines are primitive types of wine that are cloudy and effervescent containing residues of substrates and fermenting yeasts and other micro-organisms (Steinkraus, 1983). *Tej* is a home processed, but commercially available honey wine. A mixture of honey and sugar may be used as major fermentable substrate. In cases where sugar is also used as a substrate, coloring is added so that the beverage attains a yellow color similar to that made from honey (Fite et al., 1991). Some *tej* makers also add different concoctions such as barks or roots of some plants or herbal ingredients to improve flavor or potency. Due to concoction, adulteration practices and possibly some other reasons, producers usually are not willing to tell about additives used and their compositions.

According to Vogel and Gobezie (1983), during the preparation of *tej*, the fermentation pot is seasoned by smoking over smoldering *Rhamnus prenoides* stems and

*Corresponding author. Tel.: +251 1 754066; fax: +251 1 755296.
E-mail address: mogessie@gmail.com (M. Ashenafi).

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olive wood. One part of honey mixed with 2–5 (v/v) parts of water is placed in the pot, covered with a cloth for 2–3 days to ferment after which wax and top scum is removed. Some portion of the must is boiled with washed and peeled *R. prenoides* and put back to the fermenting must. The pot is covered and fermented continuously for another 5 days, in warmer weathers, or for 15–20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and *R. prenoides*.

Good quality *tej* is yellow, sweet, effervescent and cloudy due to the content of yeasts. The flavor of *tej* depends upon the part of the country where the bees have collected the nectar and the climate (Vogel and Gobezie, 1983). *Tej* is a household commercial product, and each production unit generally sells its product for consumption at point of production.

Fermentation of *tej*, like other traditionally fermented alcoholic beverages, relies on the micro-organisms present in the substrates, fermentation vats or equipment. The lactic acid bacteria (LAB) are known to produce a variety of chemical compounds relative to fermentation conditions. Their metabolic products contribute to the acidity as well as adding distinctive flavor and aroma to the fermented material. Yeasts of the genus *Saccharomyces* were reported to be responsible generally for the conversion of sugars to ethanol in *tej* (Vogel and Gobezie, 1983). However, as *tej* fermentation is a natural fermentation, variability in lactic acid and yeast flora may result in variability in acidity, flavor and alcohol content of the product. The aim of this study was, therefore, to evaluate the microbiological variability within and among commercial *tej* producing units and to assess the extent of the variability at the time *tej* was ready for consumption.

2. Materials and methods

2.1. Sampling

A total of ten *tej* producing houses from different locations in Addis Ababa were considered in this study. The production units were grouped into three based on the prices of the products. Two production units sold their products at high prices (USD 1–1.30/l), five at medium prices (about USD 0.7/l) and three at low prices (about USD 0.2/l). Selection of a particular production unit was also based on willingness of vendors to sell their products for laboratory analysis purposes.

Twenty samples (1 l each) were separately collected from each sampling site in 20 different production days. Samples were collected in sterile screw cap bottles as soon as the products were made available for consumption and were immediately brought to the laboratory for microbiological analyses.

2.2. Microbiological analyses

Twenty-five milliliters of *tej* samples were mixed with 225 ml of maximum recovery diluent (Roberts et al., 1995) and appropriate dilutions were plated on various media to count the following. Maximum Recovery Diluent is of isotonic strength to ensure recovery of organisms from various sources.

Aerobic mesophilic bacteria: A volume of 1.0 ml of appropriate dilution was pour plated on Nutrient Agar (Difco) and incubated at 30–32 °C for 48 h for counting.

Aerobic spores: Initial dilution was heat-treated at 80 °C for 10 min to kill vegetative cells. One milliliter of appropriate dilution was pour plated on Nutrient Agar and incubated at 30–32 °C for 48 h for counting.

Coliforms: A volume of 1.0 ml of appropriate dilution was pour plated on McConkey Agar No. 3 (Oxoid). The plates were incubated at 30–32 °C for 24 h to count for pink colonies.

Enterobacteriaceae: A volume of 1.0 ml of appropriate dilution was pour plated on McConkey Agar to which 10 g glucose per liter was added. The plates were incubated at 30–32 °C for 24 h to count for pink colonies.

LAB: A volume of 0.1 ml of the appropriate dilutions was spread-plated on predried surfaces of MRS agar and incubated under anaerobic conditions at 30–32 °C for 48 h.

Yeasts: Volumes of 0.1 ml of appropriate dilutions were spread plated on Chloramphenicol-Bromophenol-blue agar consisting of (g/l distilled water) yeasts extract (Oxoid) 5.0, glucose 20, choramphenicol 0.1, Bromophenol-blue 0.01, agar 15, pH 6.0–6.4. The plates were incubated at 25–28 °C for 4–5 days. Smooth, non-hairy colonies lacking extensions at margins under stereoscopic microscope were counted as yeasts.

2.3. Grouping of lactic acid bacteria (LAB)

About 20 colonies were randomly picked from countable MRS plates and purified by repeated plating. These were considered to constitute the dominant LAB flora. The isolates were studied under the microscope for their cell shape, cell grouping and presence/absence of endospores. They were also studied for their Gram reaction and catalase production. Isolates that were Gram-positive, catalase negative, non-sporing cocci- or rod-shaped bacteria were considered as LAB.

These were then grouped as homofermentative or heterofermentative groups by their ability to produce gas in 5% glucose in MRS broth after incubation at 30–32 °C for 4–5 days. Streptococci and pediococci were grossly differentiated by their cell shape, and heterofermentative cocci were considered as *Leuconostoc*. All rods (homo- and heterofermentative) were lactobacilli.