ABSTRACT

Coconut sap fermentation industry is one of the potable alcohol producing industries in the world where no systemetic studies have been done to understand microbiology and biochemistry of the fermentation process. This study reports the identity of the micro-organisms associated with the changes in physical and chemical properties of the coconut sap ('toddy') during fermentation under natural conditions.

The micro-organisms that colonizes the sap when a sterile pot was introduced to collect the sap were isolated by sampling the sap at intervals. Direct microscopic counts of the yeasts and bacteria were made to assess their distribution in the sap. High bacterial counts of 10^6 per ml. and 10^8 per ml. were observed during the first 10 hours and after 140 hours. High yeasts counts were observed between 10-140 hours reaching a maximum of 10^8 per ml.

The samples were inoculated by streak plate technique in different microbiological media for the isolation of the pure cultures. In the identification of the micro-organisms, most of the yeasts showed deviations from the standard descriptions in two biochemical reactions, the positive fermentation of sucrose and growth at $37^{\circ}C$. These were explained as adaptations to the raw material, sucrose in the sap and the tropical temperature. The reactions shown by two

sets of cultures did not fit into any of the standard descriptions. These isolates may, perhaps be new strains or substrains of yeasts that have evolved under tropical conditions.

A pattern of dominance by certain micro-organisms at certain stages of fermentation was observed. The bacteria were present at all stages of fermentation. The lactic acid producing bacteria were found to be dominant during the initial 10 hours of fermentation. The Acetobacter species were present in recognizable numbers after 140 hours of fermentation. The middle phase was dominated by yeasts. Of the yeasts Saccharomyces chevalieri was observed throughout and was present in high numbers around 100 hours. This corresponded to the stage where highest effervescence was noted in the sap. Yeasts belonging to 16 other species were isolated from the sap. Among the 166 isolates of yeasts identified the Saccharomyces chevalieri represented 35 percent of the population.

The qualitative estimation of sugars in the sap by paper chromatography showed only sucrose, fructose and glucose. Among the organic acids tested for lactic acid and acetic acid were observed regularly. Traces of succinic acid were detected occasionally.

Among the physical and chemical properties studied quantitatively the pH showed a sharp drop

from 7 to 4 within 10 hours. The specific gravity and the total sugars dropped in two stages. The reducing sugars and the ethanol showed maxima at 30 and 130 hours respectively. The acidity remained more or less constant throughout the period. The same pattern of peaks with differences in the time intervals was observed in repeating the experiment.

Based on the pattern of dominance of the microbial populations and the varying pattern of the physical and chemical properties a three stage fermentation process is suggested. A lactic fermentation occurred initially. This was followed by alcoholic (ethanolic) fermentation and an acetic fermentation. The three stages were brought about by a succession of micro-organisms.

The isolated yeasts were next tested individually for their ability to produce ethanol from sterile coconut sap. The isolates of Saccharomyces chevalieri showed a mean ethanol production of 9 percent (v/v) with a standard deviation of $\pm$ 0.62. Among the other isolates only the strains belonging to species Pichia ohmeri, Schizosaccharomyces pombe and Saccharomycodes ludwigii produced recognizable levels of ethanol.