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Section D

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MICROBIOLOGY OF COCONUT SAP FERMENTATION

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The coconut sap, an exudate obtained by “tapping” (Nathanael, 1966) the unopened inflorescence of the coconut palm, is the major raw material used in the manufacture of alcoholic beverages, toddy and arrack in Sri Lanka. The sap is a brownish clear liquid, containing 15 to 18 percent sugar almost totally in the form of sucrose. The coconut sap oozing out of the treated inflorescence, is traditionally collected in earthenware pots and allowed to ferment in open pots for varying lengths of time. During this period micro-organisms from the atmosphere enter the pot, multiply in the sugar liquid, and transform the raw materials into various products, mainly alcohol. The resulting solution containing about 7 percent alcohol is either consumed directly as toddy or distilled to obtain the spirit, arrack.

The types of micro-organisms present in a fermenting pot could vary as they are contributed from the environment. The microorganisms have the potential of producing many chemical compounds in addition to ethanol. Some of them, produced in considerable quantities, cause marked changes in the flavour of sap. Many are produced in traces. As a result, the quality of the fermented sap vary widely depending on the combination of microorganisms active in the pot.

Several reports are available on the microflora of fermenting palm wines. Most of these investigations are limited to the identification of yeasts from representative samples of the fermenting sap. Jayatissa *et al* (1978) isolated 17 strains of yeasts from coconut palm wine. The yeasts belonged to 5 genera ; *Saccharomyces* (10 strains), *Pichia* (2 strains), *Torulopsis* (2 strains), *Candida* (2 strains) and *Wingea* (1 strain), In the Philippines 40–90 percent of the yeast cultures isolated from coconut sap was found to be *Saccharomyces* sp. (Yamaga, 1980). Ahmed (1954) working on palmyrah toddy isolated *Saccharomyces chevalieri*, *Schizosaccharomyces pombe* and *Saccharomycodes ludwigii*. In the Nigerian palm wines, species of *Saccharomyces* and *Candida* are reported to be the dominant genera in the fermenting sap (Okafor, 1978).

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In this study we carefully followed some of the microbiological and biochemical changes occurring in the coconut sap during natural fermentation. The sap was allowed to collect in flame sterilized earthenware pots (as done traditionally). The sap was sampled at regular intervals of time, from the pot and subjected to microbiological and biochemical analysis. These samples were plated in different culture media to obtain as much microorganisms as possible. The isolated microorganisms were characterized (Buchnan and Gibbons, 1974 ; Lodder, 1974) and identified using commonly accepted methods. The physical and chemical characteristics such as pH, specific gravity, sugar content, alcohol level and acidity were estimated parallel with the culturing of samples (Fig. 1).

Observations

At the start, as the sap began to ooze out, it was a yellowish brown clear liquid. The pH was 7 and the sugar content was about 15 percent. The pH of the sap commenced to drop by the fourth hour, showed a rapid drop between 6–10 hours, and reached a constant value of 3.8–4.0 in a day. The drop in sugar content occurred in two stages. The total sugar dropped by about 2 percent and remained constant for about 15 hours before a second drop, which commenced around the 30th hour (Fig. 1). The second drop in the sugar content was due to the conversion of sugar into alcohol. The invert sugar reached the maximum in about 30 hours. No alcohol was produced in the first 20 hours. Alcohol was produced rapidly after 30 hours reaching a maximum in about 5 days. The alcohol dropped after the fifth day due to acetification.

Dominance by three different groups of microorganisms was noted during different stages of fermentation of sap. The biochemical changes observed in the sap could also be broadly divided into three phases, which were associated with the three groups of microorganisms.

The first phase of fermentation was dominated by bacteria. The bacterial population were of the order of 10^8 per ml. whereas the yeast population were below 10^4 per ml. The bacteria consisted of species of *Leuconostoc*, *Lactobacilli*, *Streptococci*, *Bacilli* and *Enterobacter*.

This phase corresponded from 0 to about 15–20 hours and was characterized by a rapid drop in pH from 7 to 4. No alcohol was produced during this period. The drop of sugar content by about 2 percent appeared to be associated with the production of up to 2.5 g/litre of lactic acid reported earlier (Samarajeewa *et al* 1981). The specific gravity also showed a sharp drop during this period.

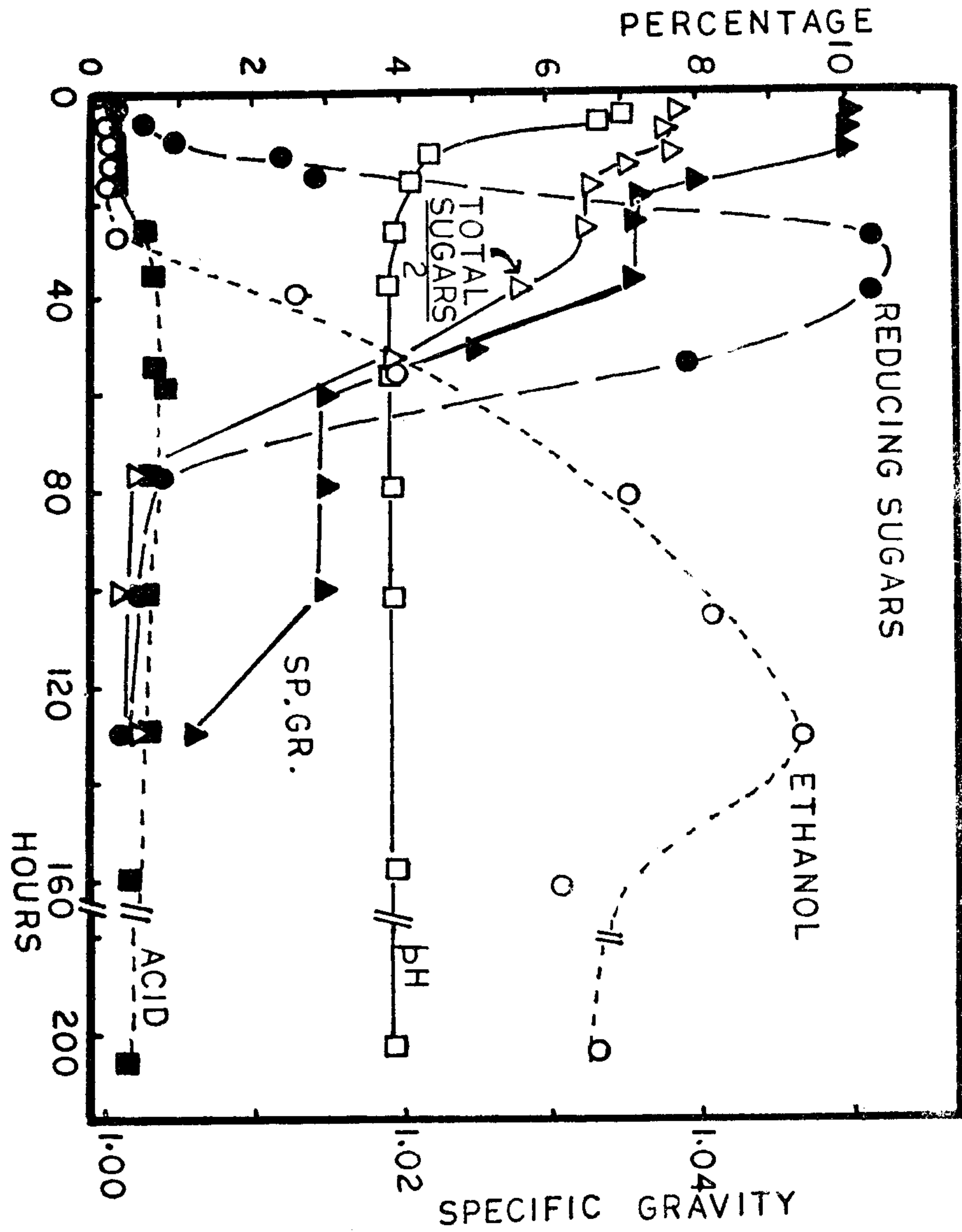


Fig. 1 Changes in Physical and Chemical parameters in coconut sap during fermentation

The second phase of fermentation was dominated by the yeasts. The yeasts identified at different hours of fermentation are shown in Table 1. Among the different species of yeasts only *Saccharomyces chevalieri* was observed in all stages of fermentation. About 35 percent of the isolates were identified as belonging to this species. *S. chevalieri* was identified in highest numbers around the 100th hour. Although several other species were detected at different stages only a few appeared to exist for long durations. Among them *Pichia ohmeri*, *Pichia membranaefaciens*, *Candida valida* and *Candida parapsilosis* were notable. Out of the above four species *Pichia ohmeri* was significant that it had similar alcohol producing capacity as *Saccharomyces chevalieri*. Most probably the other species did not survive for a long period as they had low tolerance of sugar at the initial stages or low tolerance of alcohol and acid at the final stages of alcoholic fermentation. Among the yeasts *Saccharomyces chevalieri* appeared to be the main alcohol producing organism in the sap.

TABLE 1
The yeasts identified at progressive steps of fermentation of coconut sap collected in a heat sterilized earthenware pot.

Time hrs.	4	7	10	13	18	28	39	54	61	79	103	127	157	Total
<i>Yeast</i>														
Sacch. Chevalieri ..	3	2	2	3	1	—	1	9	1	8	20	7	1	58
Sacch. bai.ii ..	1	—	—	—	—	—	—	—	—	—	—	—	—	1
Candida parapsilosis ..	1	1	2	3	1	—	—	1	—	1	—	—	—	10
Candida valida ..	—	2	—	—	1	1	1	—	2	4	—	—	1	12
Candida tropicalis ..	—	—	—	—	—	4	1	—	—	1	—	—	—	6
Candida guilliermondii ..	—	—	—	—	—	—	—	—	—	1	3	2	2	8
Pichia membranaefaciens ..	—	—	—	—	2	1	2	1	2	2	—	—	—	10
Pichia ohmeri ..	—	—	—	2	3	1	3	3	—	—	—	—	—	12
Pichia etche'sii ..	—	—	—	—	1	—	—	—	—	—	—	—	—	1
Pichia guilliermondii ..	—	—	—	—	—	—	—	—	—	—	—	1	—	1
Pichia farinosa ..	—	—	—	—	—	—	1	—	—	—	—	—	—	1
Schizo. pombe ..	—	—	1	1	—	—	—	—	—	1	1	1	—	5
Sacch. ludwigii ..	—	—	—	—	1	—	2	—	—	—	1	2	—	6
Kloeckera javanica ..	2	1	—	—	—	—	2	2	—	—	—	—	—	7
Torulopsis candida ..	—	—	—	—	—	—	—	—	—	—	1	2	1	4
Rhodotorula glutinis ..	—	—	—	—	—	—	—	1	—	1	—	—	—	2
Sporo. salmonicolor ..	—	—	—	—	—	1	—	—	—	—	—	—	—	1
Yeast like fungi ..	—	—	—	3	5	2	—	—	—	—	—	—	—	10
A ..	—	—	—	1	2	3	1	—	—	—	—	—	—	7
B ..	—	—	—	—	2	2	—	—	—	—	—	—	—	4
Total ..	7	6	5	13	19	15	14	17	5	19	26	15	5	166

This phase was characterized by a constant pH of 4. The specific gravity and the total sugar dropped again. The invert sugar reached the maximum during the initial stages (30 hours) of this phase and dropped with the commencement of alcoholic fermentation. The alcohol content increased rapidly and either remained constant or dropped slowly. The acidity remained more or less constant. This was the main phase of alcoholic fermentation.

The third phase was dominated by the acetic acid bacteria. This was a slow phase which began after 2 to 3 weeks under the conditions of our experiment.

Conclusion

The process of natural fermentation in coconut sap consisted of three different phases; a lactic fermentation, an alcoholic fermentation and an acetic fermentation. The time gaps for the three steps varied depending on the flow of sap, the microbial populations available in the pot and the time interval of removal of sap. In a normal toddy collecting pot different groups of microbes bringing about these changes were present together. Thus the three phases overlapped with each other.

The activities brought about by the microorganisms of early phases helped the activity of the microorganisms in each of the later phases. The reduction of pH by the lactic acid bacteria in the initial stages enhanced the invertase activity of the yeasts. The alcohol produced by the yeast was the raw material for the acetic acid bacteria to produce acid.

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