

EXPERIMENTAL STUDIES ON THE FERMENTATION OF ARAVINDASAVA

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ABSTRACT: *Aravindasava was prepared as per the textual and modified methods. The modified methods involved the use of glass vessel and inoculation of the autoclaved drug with the yeasts isolated from Dasamularista and Pippaliasava.*

The quantity of alcohol produced in the glass vessel was more than that in the earthen pot by classical method. Among the inoculated organisms, Dasamularista yeast II showed highest alcohol production. There was difference in the degree of utilization of reducing and non – reducing sugars by the growing microorganisms in the fermenting vessel in different method of preparation.

INTRODUCTION

Asavas and aristas are compound preparations. The preparation of asava involves the mixing of the powdered drugs, jaggery or honey or both in plant juices or water whereas in aristas a decoction of some plant ingredients is prepared, filtered, cooled and mixed with powdered drugs, honey or jaggery or both. These solutions are poured into an earthen pot coated with a layer of ghee and fumigated with pippali. The pots are closed with lid, covered with cloth, sealed with mud and are buried in the ground for a period of one month. During this period alcoholic fermentation is expected to occur.

It has been observed that quite a few microorganisms grow in the medium beside

the alcohol fermenting organism(s) (Alam *et al* 1975, 1977, 1979, 1984).

A variety of organisms have been isolated by Alam *et al* (1977, 1984) from these medicines which caused alcoholic fermentation on inoculation in other drugs (Alam *et al* 1978, 1983). Since the metabolism of each species differs, the degradation products of sugar and other compounds in these drugs will be different in the finished medicines.

The present paper describes the sugar metabolism of the yeasts isolated from Dasamularista and Pippaliasavam on their inoculation in Aravindasava. The reducing and non-reducing sugars and alcohol

produced are compared with these parameters observed in the traditional product made in accordance with the textual (classical) procedures.

MATERIALS AND METHODS:

Preparation of aravindasava:

Pure and botanically determined ingredients were used in the preparation of Aravindasava. The method of preparation was as per the Ayurvedic Formulary Part – I, Government of India (Anonymous, 1978).

The drug mixed solution was taken in an earthen pot which was coated with a layer of ghee and fumigated with pippali. The pot was closed with lid, sealed with mud and buried in sand upto neck.

The drug mixed solution was also taken in a glass flask, closed with cotton plug and kept at room temperature.

Microbiology:

Dasamula yeast I & II were isolated from Dasamularista (Alam *et al* 1984) and pippali yeast was isolated from Pippaliasava (Alam *et al* 1984). The cultures were maintained on malt extract medium at 4°C in a refrigerator and were transferred monthly to fresh slants.

Inoculunt:

10 to 15 colonies each of dasamula yeast I, II and pippali yeasts were transferred to 100 ml autoclaved Aravindasava in separate flasks and incubated at room temperature.

There was vigorous bubbling on 7th day. This solution was used as inoculum.

Pilot fermentation:

1.5 litre of Aravindasava was taken in a culture flask of 5 litre capacity and was autoclaved at 15 lbs for 15 minutes. Each autoclaved Aravindasava flask was inoculated separately with pure cultures of dasamula yeast I, II and pippali yeast.

Microbiological Screening:

A loopful of the drug from each vessel before filtration was inoculated on nutrient agar and incubated at room temperature for 72 hours. The organisms that showed up were isolated and identified. The staining procedures used for the identification of the organisms were those described in literature (Peltier *et al* 1955).

Analytical methods:

pH was determined on Elico pH meter, Specific gravity, solid content, reducing and non – reducing sugar and alcohol were determined as reported earlier (Alam *et al* 1979).

RESULTS

The pH of Aravindasava before fermentation was 4.94 and after fermentation 5.51 in earthen pot and 5.39 in glass vessel. The glass vessel pH was comparable to that of Dasamula I and Pippali yeast fermented medicines (Table I & II)

TABLE - I

Analytical values of fermented Aravindasava prepared in earthen pot and glass vessel by classical method

Parameter	Before fermentation	Earthen pot	Glass vessel
pH	4.94	5.51	5.39
Specific gravity	1.088	1.006	1.007
Solid content % (W/W)	22.93	4.48	4.99
Reducing sugar % (W/ W)	7.23	1.30	1.45
Non-reducing sugar % (W/W)	11.95	0.49	0.11
Total sugar % (W/W)	19.18	1.79	1.57
Alcohol % (V/V)	-	10.05	10.9

TABLE – II

Analytical values of fermented Aravindasava autoclaved and inoculated with different yeasts

Parameter	Dasamula yeast I	Dasamula yeast II	Pippali yeast
pH	5.24	5.38	5.41
Specific gravity	1.019	1.012	1.035
Solid content % (W/W)	6.90	6.74	11.15
Reducing sugar % (W/ W)	4.24	2.42	0.81
Non-reducing sugar % (W/W)	0.17	0.05	6.86
Total sugar % (W/W)	4.41	2.47	7.67
Alcohol % (V/V)	8.05	10.52	5.90

The specific gravity of the drug before fermentation was 1.088 whereas after fermentation it was 1.006 in earthen pot and 1.007 in glass vessel. With the inoculated organisms, dasamulat yeast I and II showed comparable specific gravity whereas with pippali yeast it was 1.035.

The solid contents were also proportional to that of specific gravity in each container (Tables I & II).

The non – reducing sugar in the medicines before fermentation was 7.23%. In the fermented drug it was 1.3% in earthen pot, 1.45% in glass vessel and 4.24%, 2.42% and 0.81% with dasamula yeast I, II and pippali yeast respectively.

The alcoholic content in earthen pot and glass vessel were 10.05% and 10.9% respectively. Among the inoculated organisms highest alcohol was produced by dasamula yeast II followed by dasamula yeast I and pippali yeast (Table II).

The content of alcohol produced in earthen pot and glass vessel was higher than the total sugar observed before fermentation. This may be due to release of sugar from draksa (*Vitis vinifera* Linn) which is one of the ingredients in this preparation. The other reason may be due to degradation of polysaccharids to glucose by the growing organisms.

The alcohol produced by the pippali yeast is corresponding to the amount of sugar consumed whereas with the dasamula yeast I the alcohol produced and the total sugar shows a difference of 1%.

The dasamula yeast II alcohol production was also no corresponding to the total sugar of the drug. Here again the excess sugar

should have resulted by the degradation of the polysaccharide or from draksa.

The microbiological of the drug after fermentation revealed *Bacillus* sp., yeast, *Aspergillus niger* and *A. Wentii* in earthen pot. The organisms in the glass vessel were *Bacillus* sp., rod shaped bacteria yeast and *Aspergillus niger*. In the finished medicine dasamula yeast was not present whereas pippali yeast was present.

DISCUSSION

The variation of pH in Aravindasava prepared in glass vessel and earthen pot may be due to the growth of different microorganisms in the fermenting vessel and also may be due to the material of the fermenting vessel. The pH of dasamula yeast II and pippali yeast drugs were comparable to the pH attained in the glass vessel thereby indicating some similarities in the metabolic activities of these organisms.

All the tested yeast caused alcoholic fermentation but to varying degrees. The quantity of alcohol produced with yeast II in glass vessel was more than that produced in earthen pot and glass vessel by classical method (Table I & II).

Sharma et al (1975) have indicated the therapeutic value of the sugar remaining in the finished product which constitutes the medicine. In Aravindasava reducing sugar was comparable in the earthen pot and glass vessel whereas non-reducing sugar was more in earthen pot (Table I). Among the inoculated organisms non – reducing sugar was higher in pippali yeast than dasamula yeast preparations. The reducing sugar was high in dasamula yeast I compared to other two yeasts (Table II).

CONCLUSION

Organisms that produced ethanol in Dasamularista and Pippliasava even performed satisfactory in Aravindasava medium also. With respect to alcoholic content the efficiency of dasamula yeast I was comparable to the level of product made by textual methods. Alam et al (1977, 1978, 1983, 1984) have also shown the fermentation of different asavas and aristas with the organisms isolated from different medicines, thus demonstrating that these organisms are versatile.

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There was variation of reducing and non-reducing sugars in different preparations. The therapeutic effect of the remaining sugar in the drug can be assessed either biologically or chemically.

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