CHAPTER - IV
MICROBIAL AND BIOCHEMICAL CHANGES DURING PRIMARY FERMENTATION OF SELECTED GRAINS

4.1. INTRODUCTION

Primary fermentation is the first step in koozh processing which involves soaking millet flour in water for 12 -15 h. Fermentation is spontaneous with respect to household preparations while with local producers backslopping is commonly performed by adding approximately 20-30 ml of 12 -15 h fermented slurry to 500 ml of gruel. Finger millet is the widely used substrate in koozh preparation and in some parts of TamilNadu, Pearl millet is also used. Primary fermentation of finger millet was reported to cause various biochemical changes in the substrate and LAB was reported as the predominant organism (Antony et al., 1996). Reports suggest that primary fermentation step induces various biochemical changes in the substrate which are of nutritional significance. Though according to traditional preparatory procedures any grain flour can be used for the preparation of koozh, till now only finger millet has been studied as a substrate for koozh fermentation.

Therefore an attempt was made to include other grains viz., Pearl millet, Maize and Sorghum in koozh preparation. The aim is to include largely available but underutilised millet species into diet. The microbial succession during primary fermentation and biochemical changes were studied. The acceptability of the koozh prepared from the grains was also analysed by sensory evaluation test.

4.2. EXPERIMENTAL INVESTIGATIONS

4.2.1. SAMPLES USED

Four different grains, commonly available in markets were chosen for this part of study. Finger millet (Eleucine corocana L.), Pearl millet (Pennisitum typhoides L), Sorghum (Sorghum bicolour L.) and Maize (Zea mays L) were purchased from local market, Vellore (Tamil Nadu, India).

4.2.2. SAMPLE PROCESSING

Grains were cleared off stone, mud and other impurities washed in running water, dried in shade, milled into flour and stored in air-tight containers at room temperature. Primary fermentation was carried out adopting traditional procedure:
25 g of the milled flour was mixed with 50 ml of water, covered with aluminium foil and incubated at room temperature. To stimulate home conditions unsterilized containers and drinking water were used for fermentation.

4.2.3. SAMPLE COLLECTION

Fermentation proceeded for 20 h and at each 5 h intervals samples were collected in sterile bottles. Microbial analysis was performed immediately after sample collection. A part of the sample was dried in hot air oven at 60 °C overnight, powdered manually in pestle and mortar. Powdered samples were used for sugar and protein analysis.

4.2.4. SAMPLE ANALYSIS

Samples were analysed for microbial succession during primary fermentation adopting procedure described in chapter 3 (section 3.2.1). pH, Titrable acidity, sugar analysis and protein changes were carried out according to the procedure described in chapter 3 (section 3.3.1, 3.3.2, 3.3.3 and 3.3.4).

4.2.5. SENSORY EVALUATION

Sensory qualities of the koozh prepared from the above mentioned grains were analysed using nine point hedonic scale. Twenty five respondents from ages 21 - 45 were involved in this test. Respondents had prior knowledge about the product. The questionnaire of the analysis was attached in Appendice No. III.

4.3. RESULTS AND DISCUSSION

The major biochemical changes during natural fermentation of the grains are represented in Figs 4.1a and 4.1b. There was a significant increase in reducing sugar concentration in finger millet and pearl millet till 10 h of fermentation while in maize and sorghum no such increase was observed. The increase in sugars in the millets may be either due to the solubilisation of sugars as water was added to the substrates (Antony et al., 1996) or due to starch hydrolysis. Concurrent to this observation there was about 2% and 3.5% decrease in starch content in finger millet and pearl millet respectively. There was no significant change in starch content of maize and sorghum during fermentation. The native maize starch was reported to be surrounded by protein molecules which prevent enzymatic hydrolysis of starch and hence forms
the reason for this observation (Wu et al., 2008). Total sugar concentration remained statistically insignificant (p<0.05) in all the grains (Fig 4.1b) implying solubilisation of carbohydrates by hydrolyzing microflora which has been documented by other workers (Sripriya et al., 1997). The changes in sugar content in finger millet and pearl millet declared the involvement of starch hydrolysing bacteria population in the fermentation process which was confirmed with microbial studies.

Microbial analysis showed that in all the grain substrates bacterial population was more than yeast. Total aerobic bacteria population was increasing throughout the fermentation period in all the grains (Fig 4.2) and comprised of starch hydrolysing bacteria and Enterobacter population. At the start of fermentation, starch hydrolysing bacterial population was numerically higher in all the grains when compared to other bacterial groups (Fig 4.3). But significant increase in population was noticed only in finger millet and pearl millet till 10 h of fermentation. In sorghum and maize, starch hydrolysing bacterial population was found to be decreasing during the fermentation period. The changes in starch hydrolysing bacterial population coincided with the sugar changes observed during fermentation of the grains. The ratio of total bacteria to starch hydrolysing bacteria at 10 h of fermentation in finger millet and pearl millet was found to be 3:1 and 2:1 respectively. The contribution of starch hydrolyzers in finger millet and pearl millet up to 10 h of fermentation was significant (r = 0.59, 0.64; p < 0.05) and thereafter it became non-significant. Till now there are only limited reports on the role of starch hydrolysers in the fermentation of grains; however numerous reports stated the role of amylolytic LAB during cereal and millet fermentation (Olasupo et al., 1996). The results of this study bring out the importance of starch hydrolyzers during koozh preparation with finger millet and pearl millet.
Fig. 4.1a Changes in reducing sugar content at every 5h interval of primary fermentation of grains. Error bars represent standard deviation of three replications.

Fig. 4.1b Changes in total sugar content at every 5h interval of primary fermentation of grains. Error bars represent standard deviation of three replications.
Fig. 4.2 Total bacteria population at different primary fermentation hours of grains
FM - Finger millet, PM- Pearl millet, MAI- Maize, SOR - Sorghum
Error bars represent standard deviation of five replications

Fig. 4.3 Total Starch hydrolysing bacteria population at different primary fermentation hours of grains
FM - Finger millet, PM - Pearl millet, MAI - Maize, SOR - Sorghum
Error bars represent standard deviation of five replications
Enterobacter population was also found to be increasing significantly in all the grains till 10 h of fermentation after which the population decreased in finger millet, maize and sorghum (Fig 4.4). But in pearl millet the population remained constant as influenced by storage conditions. Enterobacter are common as seed microflora and was reported to be present in many cereal fermentations (Adegoke and Babalola, 1988) but LAB population reported to inhibit their growth during cereal and millet fermentation (Sulma et al., 1991). Likewise in koozh fermentation the decrease of starch hydrolysing bacteria and Enterobacter during the later hours of fermentation was attributed to the dominance of LAB population (Fig 4.5).

LAB become the dominant flora during the later hours of fermentation (10 - 20h) in all the grains. The sugars made available in the substrates can be stated as the reason for the rise in LAB population after 10 h of fermentation. All the tested grains were found to be suitable substrates for LAB as noticed by the significant increase in the count till 20 h of fermentation. No significant variations in the LAB count were observed among the tested grains. LAB generally was reported as the predominant microflora in various traditional cereals and millet based fermented foods (Nour et al., 2004, Omemu et al., 2007). Due to LAB succession there was a significant reduction in pH in all the substrates after 10 h of fermentation. There was about two units decrease in pH in all the grains with concomitant increase in acidity (Table 4.1). Significant negative correlation ($r = -0.927; p < 0.05$) existed between fermentation time and pH. Coliforms were not observed in any of the grains during fermentation. Along with acid production LAB strains were known to produce proteinaceous compounds which were effective against coliforms as reported by Omar et al., (2006).

Compared to bacterial population, yeast population was low in all the grains and was found only after 15 h of fermentation in the substrates. The results were in agreement with the reports of Antony et al., (1997) in finger millet fermentation. But this is in sharp contrast to other fermentations like kenkey, mawe and mahewu (Hounhouigan et al., 1993, Jespersen et al., 1994) prepared from sorghum and maize. The lower population of yeasts in grains could be due to the unfavourable pH during initial hours.
Fig. 4.4 Total Enterobacter population at different primary fermentation hours of grains
FM - Finger millet, PM - Pearl millet, MAI - Maize, SOR - Sorghum
Error bars represent standard deviation of five replications

Fig. 4.5 LAB population at different primary fermentation hours of grains
FM - Finger millet, PM - Pearl millet, MAI - Maize, SOR - Sorghum
Error bar represent standard deviation of five replications
Table 4.1

Changes in pH and titrable acidity during different primary fermentation hours of grains

<table>
<thead>
<tr>
<th>Fermentation hour (h)</th>
<th>Finger millet</th>
<th>Pearl millet</th>
<th>Maize</th>
<th>Sorghum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.2 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.36 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>6 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.1 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.16 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>5.7 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.76 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>5.1 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.5 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.2 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>4.8 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.53 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.3 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.9 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- T.A. represents titrable acidity and the values are expressed as volume of NaOH consumed to neutralize acid present in 1 ml of sample.
- Value are mean of triplicate samples (n=3±SD). Means not sharing common letter in a column are significantly different from each other as assessed by Duncan multiple range tests.
Yeast population was expected at the later stages of fermentation when sugars were made available but the short duration of primary fermentation might have prevented their propagation.

Other than sugar changes microbial population also brought significant changes in protein content of the grains (Fig. 4.6). Significant rise in protein concentration was observed in all the grains at 10-15 h fermentation period which indicates the proteolytic activities of LAB population as they were the dominant group during that period. These results go on par with Elyas et al., (2002) where natural fermentation of pearl millet reported to increase protein availability in the substrate. According to Zamora and Fields (1979) increased protein content can be attributed to the microbial synthesis of proteins from metabolic intermediates during their growth cycles.

Ethanol production was also observed during fermentation in all the grain substrates. But there were no significant changes in the concentration during fermentation (Fig 4.7). As yeast population was low in all the substrates there is a possibility that heterofermentative LAB population may have contributed for ethanol production.

Fermentation was repeated in five different batches and the results obtained showed similar microbial profile in all the grains. This confirms the involvement of endogenous microflora of the grains. According to Semeniuk (1954) microorganisms involved in natural fermentation of cereals are essentially the surface microflora of seeds. Fungal growth was not found in any of the grains during the observed period. Lactic acid fermentation was well known to reduce fungi population in cereal substrates and its toxin production. Also tannin content in the grains restricts fungal growth as stated by Riley et al. (1993). Thus the observations have shown that though microbial composition was similar among the grains, variations in the population affected biochemical changes in the substrate which influenced sensory properties of the product.
Fig 4.6 Changes in protein content at different primary fermentation hours of grains
Error bars represent standard deviation of three replications.

Fig 4.7. Changes in ethanol concentration at different primary fermentation hours of grains
Error bars represent standard deviation of three replications.
Fig 4.8 Organoleptic evaluation of fermented grain substrates.
I- Overall acceptability; II- Flavour III- Taste, IV- Appearance
Primary fermented millet flours after thermal treatment were tested for its acceptability and sensory qualities. Finger millet koozh was highly acceptable in taste and flavour followed by pearl millet (Fig 4.8). Sensory scores for flavour and taste were highest for finger millet when compared with other grains. This may be due to softening of the substrate by starch hydrolysing microorganisms as amylolytic activity was reported to improve the rheology of the product (Ojijo and Shimoni, 2004). This again emphasise the importance of starch hydrolysing bacteria population in koozh fermentation process.

Koozh prepared from maize and sorghum was unacceptable by panel members as they strongly felt that the product may cause indigestion. Hence the use of starch hydrolysing organisms as starter culture will help in effective starch hydrolysis and may improve digestibility and flavour of the product. Also change in sample processing such as decortication of the grains or wet milling prior to primary fermentation will also help in improved starch hydrolysis thus enhance the sensory qualities of the product.

4.4. CONCLUSION

Based on the results obtained from sensory tests and considering wide usage and previous published results finger millet was selected for secondary fermentation studies.