

A study on the fermentation pattern of common millets in *Koozh* preparation – a traditional South Indian food

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Received 10.10.11, revised 10.10.11

Koozh is a popular South Indian traditional food made from finger millet (*Eleusine corocana* Gaertn.). The traditional preparation method was applied to other common millets, viz. Pearl millet [*Pennisetum typhoides* (Burm.f.) Stapf & C.E. Hubbard], Sorghum (*Sorghum bicolor* (L.) Moench) and Maize (*Zea mays* L.). The fermentation process was monitored for microbial succession. The biochemical changes and sensory properties were evaluated and compared with traditional finger millet fermentation process. Microbial profile and biochemical changes in selected millets were identical to traditional fermentation process. Starch hydrolases were the primary players in the initial hours (0 -10 hrs) of fermentation in all grains, while lactic acid bacteria (LAB) dominated the later hours (0 – 15 hrs). Starch hydrolysis was the major biochemical transformation occurred during the initial fermentation period, and in later conversion of sugar into acids took place which made the medium acidic. *Koozh* prepared from pearl millet and sorghum had sharp flavor and received acceptability from consumers, while Maize *Koozh* did not appeal to the consumers. This study is an attempt to add variety to traditional process and to improve commercial value and marketability.

Keywords: Porridge fermentation, Lactic acid bacteria, Yeast, *Enterobacter*, Starch hydrolases, Finger millet, Pearl millet, Sorghum, Maize

IPC Int. Cl.⁸: A61K 36/00, A47G 19/26, A47J 39/02, A23, A21, C12N 1/16- C12N 1/18, C12M, C12N, C12N 1/00

Millets were used as staple food for thousands of years by people in Asian and African countries before rice became a common commodity to man. Various millet based traditional preparations are available throughout the world and fermentation of millets is a common practice¹. Fermentation has a positive influence on grains^{2, 3, 4}, and also inevitable with regard to millet preparations, which is well recognized and accepted from ancient times. Traditional fermented foods have received extensive scientific attention and many traditional preparations have been analyzed for their microbiological, enzymological and biochemical changes^{5, 6, 7}. Almost all traditional preparations start with natural fermentation involving mixed microbial cultures. Previous reports on fermentation of grains stated major transformations occurring during the process, viz. starch hydrolysis, sugar transformation, improvement of protein, mineral availability^{8, 9} and grain softening^{10, 11, 12}. In South India, among the five

common millets, finger millet is commonly consumed and others are not usually included in the diet.

One of the popular traditional finger millet preparations in south India is *Koozh* which has long history and is mainly associated with the local population. This fermented food was best known for its flavor and nutritive value, so it was regularly used as a breakfast meal which was then slowly replaced by rice based foods. Now, it is again becoming popular, as an alternative carbohydrate source for rice and wheat. Hence, *Koozh* preparation has been started as a small scale business and only finger millet is commonly used as raw material. This study is an attempt to include other millets, viz. Pearl millet, Maize and Sorghum in *koozh* preparation with the aim of adding variety, nutritive quality and commercial value to *koozh* market. The preparatory method of *koozh* is unique which involves two fermentation stages. Primary fermentation of *koozh* was done for 12 – 15 hrs which forms the basis for the product quality and sensory properties. Hence, in this study comparative analysis was done on the microbial and

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biochemical changes during primary fermentation of the chosen millets with the already existing finger millet porridge. The acceptability of the *koozh* prepared from other millets was also investigated.

Materials and Methods

All the chemicals and reagents used in the study were from Hi Media, Mumbai and Sigma Chemicals, Bangalore. The chemicals were all of analytical grade.

Finger millet, pearl millet, sorghum and maize were purchased from local market, Vellore (Tamil Nadu, India). The millets 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 until use. Natural fermentation was carried out by mixing 25 gm of the milled flour in water in the ratio of 1:2. De-ionized water was used for fermentation.

Samples were collected at every 5 hrs interval till 20 hrs in sterile glass vials and if necessary stored at 4°C for analysis.

Changes in pH during fermentation were monitored at every 5 hrs intervals with a pH meter (Systronics, Chennai) after calibrating with standard buffers. Titrable acidity of the sample was determined by titrating 1 ml of sample with 0.1N NaOH with phenolphthalein as indicator⁶.

For sugar analysis, one gm of sample was sonicated for 2 min followed by centrifugation at 8000 rpm for 10 min. The supernatant was analyzed for total sugars by phenol-sulphuric acid method¹³ and for reducing sugars by DNSA method¹⁴ using glucose as standard. The same aliquots were used for protein analysis by Lowry's method¹⁵ with BSA as standard.

Microbial analysis was carried out as follows: 1 gm of the sample was dissolved in sterile saline (0.84% NaCl) for serial dilutions. Nutrient agar media was used for enumeration of total aerobic bacteria with 24 hrs incubation period at 37°C. For the enumeration of lactic acid bacteria MRS agar medium was used. Distinct colonies were selected based on morphology and maintained in MRS agar plates for further characterization. Enumeration of yeasts was done in YPD agar supplemented with 0.3 mg/ 100 ml of chloramphenicol. *Enterobacter* was enumerated in EMB agar. Enumeration of starch-degrading organisms was performed by testing starch hydrolysis in nutrient agar media containing 1% starch.

Sensory qualities of the *koozh* prepared from the above mentioned grains were compared using

9 point hedonic scale. Twenty five respondents of all age groups from 21 - 45 yrs were involved for this test. Respondents had prior knowledge about the product.

All biochemical analyses were done in triplicates. Microbiological analyses consisted of three separate samples of each grain in triplicates. Data obtained were analyzed for mean, standard deviation, correlation, ANOVA, and critical mean differences using Microsoft Excel 2003 (Microsoft Inc., USA) and Sigma Plot version 10 software (StatSoft Inc, USA).

Results and Discussion

The major biochemical changes during natural fermentation of the millets are represented in Figs. 1(a-c). Traditional fermentation process in finger millet witnessed major biochemical changes from 5 - 15 hrs indicating a window period of 10 hrs. The trend with respect to sugar changes was similar in all the tested grains. During traditional finger millet fermentation process, significant increase in reducing sugar concentration was observed up to 10 hrs (Fig. 1a). Similar increase in reducing sugar concentration was observed in other grains also. Starch hydrolysis was found to be the major biochemical transformation occurred during primary fermentation irrespective of the grain type. These results are in agreement with studies conducted on other millets, viz. fox tail millet fermentation¹⁶, where 10% reduction in starch was reported. Among the millets, pearl millet grains had significant reducing sugar content probably due to easily digestible starch type. Total sugar concentration remained statistically insignificant in all the grains during fermentation implying solubilisation of carbohydrates by hydrolyzing microflora which has been documented by other workers¹⁷. This was, also shown by the higher activities of aerobic bacteria and starch hydrolysing organisms [Figs. 2(a -b)] during the first 10 hrs in all the grains tested.

In traditional finger millet fermentation process, total aerobic bacteria and starch hydrolysing bacteria were playing major role during initial hours of primary fermentation (up to 10 hrs). Total bacterial count increased throughout the fermentation time, starch hydrolysing bacteria population declined after 10 hrs. Other tested millets also had similar microbial profile during the initial hours of fermentation and there was no significant difference in the count among

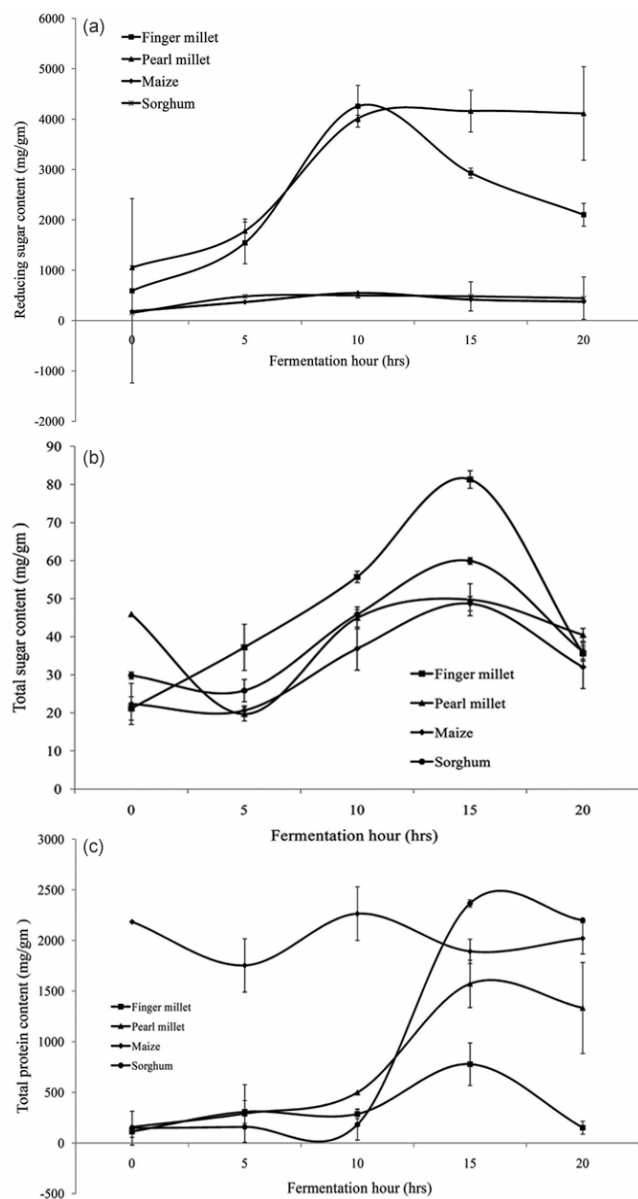


Fig. 1(a-c)—(a) Change in reducing sugar content at every 5hrs interval of fermentation of millets; (b) Change in total sugar content at every 5hrs interval of fermentation of millets; (c) Change in protein content of millets during fermentation.

the tested grains and finger millet. With regard to starch hydrolysers significant increase in count was observed in traditional fermentation till 10 hrs, and in Maize till 5 hrs after which decline in population was recorded.

Till now, there are only limited reports on the role of starch hydrolysers in the fermentation of grains; however other findings had stated the role of amylolytic lactic acid bacteria during fermentation³. The results of this study bring out the importance of

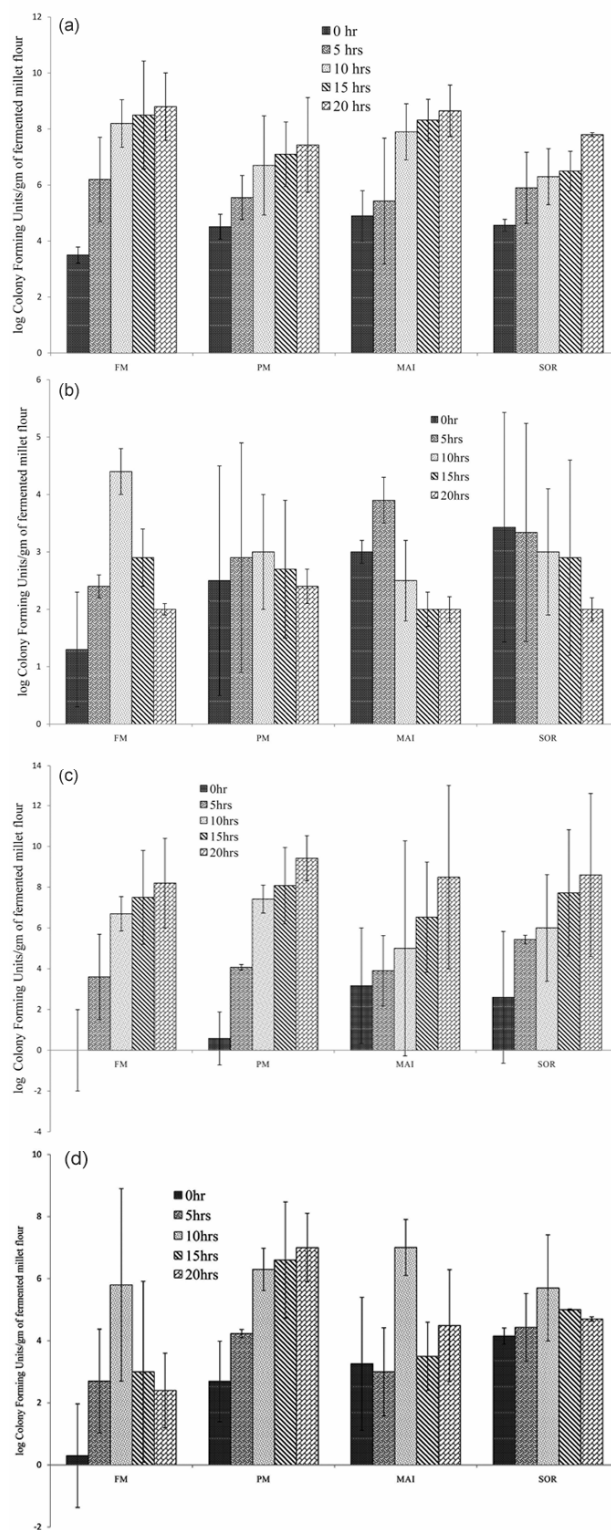


Fig. 2(a-d)—(a) Total bacterial population at different fermentation hours of tested millets; (b) Total Starch hydrolysers population at different fermentation hours of millets; (c) Lactic acid bacterial population at different fermentation hours of tested millets; (d) Total Enterobacter count at different fermentation hours of millets.

starch hydrolysers during *koozh* preparation. Also the activity of starch hydrolysers was found to be influenced by the grain type. Next to starch hydrolysers, total lactic acid bacteria population was dominating the fermentation process in finger millet fermentation (Fig. 2c). Two way ANOVA test ($p < 0.05$) indicated significant increase in lactic acid bacteria population after 10 hrs of fermentation. This also coincided with the maximum available reducing sugars by the activity of starch hydrolyzers making lactic acid bacteria as primary successors. All the tested millets were found to be suitable substrates for lactic acid bacteria as evidenced by the significant increase in the count till 20 hrs. The involvement of lactic acid bacteria in cereal based preparations has been well documented^{6, 7}. No significant variation in the lactic acid bacterial count among the tested grains was found.

The activity of the lactic acid bacteria in the later hours of fermentation brought down the pH significantly and increased acid accumulation in the medium (Table 1). Significant negative correlation ($r = -0.927$; $p < 0.05$) existed between fermentation time and pH during traditional finger millet fermentation. Similar changes were recorded in other grains also. In finger millet processing, 1.4 units decrease in pH was noted; in pearl millet 1.9 units in maize and sorghum 1.7 units and 1.6 units, respectively. These results are in accordance with other reports on fermented cereal preparations^{18, 19}. In pearl millet and sorghum, significant decrease in pH was recorded from 10 hrs, while in maize and finger millet it was from 15 hrs. A decrease in pH may be one of the reasons for the decline in the population of starch hydrolysers. Since the pH drop was more in pearl millet, starch hydrolysers count was also less compared with other millets.

There was no significant change in protein content during finger millet fermentation (Fig. 1c). But in sorghum and pearl millet significant increase in protein content was recorded at 15 hrs of fermentation as already documented²⁰ whereas in maize there was no change in the protein content during fermentation.

Other microbial groups involved in the fermentation process involved in finger millet fermentation were *Enterobacter* (Fig. 2d) and yeasts. A steady increase in *Enterobacter* count was observed till 10 hrs, after which decline in microbial population was noticed. In maize and sorghum, similar results were obtained. But in pearl millet population remained constant. Similar results were observed by²¹ in *Kisra* fermentation where *Enterobacter* were suppressed after 24 hrs because of the dominance of other groups. However, there are reports on equal contribution of *Enterobacter* along with lactic acid bacteria in fermentation²², but in *koozh* preparation their contribution was not significant. Coliforms were not detected during fermentation. Unlike other microbial groups, *Enterobacter* count was strongly influenced by grain type indicating that source of grains may play an important role.

Yeast population was found to be low throughout fermentation till 20 hrs. This is in sharp contrast to other fermentations like *kenkey*, *mawe* and *mahewu*^{23, 24}. In all the grains, there existed a positive correlation among the heterotrophic bacteria, lactic acid bacteria and *Enterobacter* but starch hydrolyzers were observed to have no influence on other population probably due to their lower numbers. The contribution of starch hydrolyzers up to 10 hrs of fermentation was significant ($r = 0.59$; $p < 0.05$) and thereafter, it became non-significant. Later changes in reducing sugar can be attributed to

Table 1—Changes in pH and titrable acidity during different fermentation hours of grains

Fermentation hour (hrs)	Finger millet		Pearl millet		Maize		Sorghum	
	pH	Titrable acidity	pH	Titrable acidity	pH	Titrable acidity	pH	Titrable acidity
0	6.2 ± 0.09 ^a	0.56 ± 0.15 ^a	6.2 ± 0.09 ^a	1.36 ± 0.2 ^a	6.4 ± 0.1 ^a	1.06 ± 0.2 ^a	6.5 ± 0.08 ^a	1.23 ± 0.2 ^a
5	6 ± 0.08 ^a	0.8 ± 0.52 ^a	6.1 ± 0.05 ^a	2.16 ± 0.05 ^a	6.0 ± .09 ^a	1.23 ± 0.15 ^a	6.4 ± 0.09 ^a	1.06 ± 0.05 ^a
10	5.7 ± 0.14 ^a	1.3 ± 0.1 ^a	5.2 ± 0.1 ^b	2.76 ± 0.2 ^a	5.8 ± 0.07 ^b	1.5 ± 0.1 ^a	5.6 ± 0.06 ^b	2.1 ± 0.1 ^a
15	5.1 ± 0.05 ^b	1.8 ± 0.11 ^b	4.5 ± 0.03 ^c	4.2 ± 0.4 ^b	5.1 ± 0.2 ^c	2.06 ± 0.11 ^b	5.3 ± 0.08 ^c	2.96 ± 0.05 ^b
20	4.8 ± 0.19 ^c	2.53 ± 0.1 ^c	4.3 ± 0.13 ^d	5.9 ± 1.4 ^c	4.7 ± 0.8 ^d	2.5 ± 0.1 ^c	4.9 ± 0.08 ^d	3.13 ± 0.15 ^c

^a Titrable acidity values are expressed as volume of NaOH consumed to neutralize acid present in 1 ml of sample

^b Values are means of triplicate samples (n=3) (± SD). Means not sharing a common letter in a column are significantly different at $p < 0.05$ as assessed by Duncan's multiple range tests.

lactic acid bacteria as they indicated a significant correlation in our study ($r = 0.45$; $p < 0.05$) and in accordance with previous studies on amylolytic lactic acid bacteria³. The lower population of yeasts in grains could be due to the unfavourable pH. Yeast population was expected at the later stages of fermentation when high sugars were made available but the short duration of primary fermentation might have prevented their propagation.

The above results have shown that the microbial profile and biochemical changes were similar irrespective of grain type during primary fermentation. But the organoleptic property of the product forms the important part. Hence, primary fermented millet flours were tested for its acceptability and sensory qualities. Next to traditional finger millet *koozh*, pearl millet *koozh* and sorghum *koozh* was acceptable in taste and flavour. *Koozh* prepared from maize was unacceptable to consumers and strongly feel it may cause indigestion if consumed. Hence, use of starch hydrolysing organisms as starter culture will help in effective digestion of starchy substance and may in improving digestibility and flavour of the product.

Conclusion

The present study has shown that primary fermentation of common grains used for *koozh* preparation includes various groups of microorganisms predominantly starch degrading ones and lactic acid bacteria. In the initial hours, starch forms the major source for microbes which evidently lead to an increase in reducing sugar content and in the later part for the conversion of sugars results in acid production. Though the microbial profile was found to be similar in all grains, protein changes depend on the composition of grains. Sorghum *koozh* and pearl millet *koozh* was found to have a good taste and sharp flavor and earned consumers interest. Maize based *koozh* preparation was not appealing; hence it can be added with millets and used. Millet based *koozh* preparations are a very good alternative for other carbohydrate-rich cereal products. *Koozh* preparations with other unused millets will add variety and marketability to the presently available preparation.

Acknowledgement

The authors thank VIT University for providing lab facilities for carrying out this work.

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