International Journal of Life Sciences
International Peer Reviewed Open Access Journal

Special Issue | A20 | April 2023
ISSN:2320-7817(p) 2320-964X(0)

Original Article Open Access

Effects of various utensils on steeped sorghum fermentation in terms of lactic acid bacteria

Khade PJ, Khopaye MP and Phirke NV *

P. G. Department of Microbiology, Sant Gadge Baba Amravati University, Amravati-444602, India,
*Corresponding author Email: phirkenv@gmail.com

Manuscript details:

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Cite this article as:
Khade PJ, Khopaye MP and Phirke NV (2023) Effects of various utensils on steeped sorghum fermentation in terms of lactic acid bacteria, Int. J. of. Life Sciences, Special Issue – A 20 : 7-12.

Article published in Special Issue on "*National conference on Advances in Biodiversity Conservation and Sustainable development: Educational & Scientific Research Perspective NCABCSD-2023*" organised by Department of Life Sciences, Shri Shivaji Science College, Amravati (MS) India - 444 603 Dated March 3 - 4, 2023.

ABSTRACT

In *Vidharbha Khandesh* region, different utensils are used in traditional fermented items. Here, we tried to identify which utensils are good for homemade Sorghum fermentation according to lactic acid bacterial population and its ecological association. The sorghum grains were soaked in water in 1:2 (w/v) proportions allowed to ferment. The colony count was measured and compared accordingly. The lactic acid bacterial count in utensils were found increasing in order of clay > aluminium > steel > glass > brass > plastic made utensils. So that in domestic use sorghum fermentation, clay utensil is good for household traditional fermented food items.

Keywords: fermentation, sorghum grains, utensils, LAB

INTRODUCTION

Sorghum is widely grown in sub-tropical regions of Africa and Asia because of its drought tolerance. It is considered the food of the poor and more than 70% of the food can be consumed by the people of this region. Numerous studies involving human, animal and *in vitro* digestion have shown that cooked sorghum has lower protein digestion than other cereals. Sorghum was found to ferment before cooking to improve *in vitro* and *in vivo* digestion. Sorghum fermentation is commonly used in Sudan to make a number of food (Mohammed *et al*., 1991; Day and Morawicki, 2018). There are many varieties of sorghum (Butti and More, 2016). Sorghum is used in the production of different food preparations such as bread, porridge, pancakes, muffins, dumplings and breakfast cereals like ogi. It contains more fat than wheat and rice, but slightly less than corn. Sorghum is a very important food crop, because it is gluten-free which makes it an excellent replacement for people that are allergic to gluten intake (Ojokoh *et al*., 2020). The fermentation process has been taking place in India for many centuries, so the taste of the food increases, its nutritional value increases and increases the shelf life of food which preserve it by using lactic acid, alcohol

and acetic acid (Abah *et al*., 2020). Different fermented food items are made in *Vidarbha* and surrounding region like *Jalebi*, *Dosa*, *Idli*, *Papad*, and *Sorghum Papad*. Our focus is on sorghum fermented food items. Various utensils are used in making sorghum fermented food items such as clay, aluminum, steel, glass, brass and plastic utensils and containers. In this study we aimed to identify which utensils are good for making traditional homemade sorghum fermented food items on the basis of microbial population in sorghum fermented batter.

MATERIALS AND METHOD

Materials: Cereals (*Sorghum* grains), MRS (deMan, Rogosa and Sharpe) agar, Glass Utensil, Plastic Utensil, Steel Utensil, Brass Utensil, Clay Utensil, Aluminium Utensil, Sterile water, Cotton Cloth, Crystal violet, Iodine, Safranin (0.25%), Alcohol (0.95%), Hydrogen peroxide (0.3%) and Oxidase reagent (1% tetramethylene paraphenylene diamine dihydrochloride).

MRS media: MRS (deMan, Rogosa and Sharpe 1960) agar, MRS broth. Composition of MRS media (Kunene *et al.,* 2000, Khade and Phirke, 2014) is shown in table no.1. Developed by de Man, Ragosa and Sharpe in 1960, MRS has been used enormously in the enumeration and isolation of *Lactobacilli*.

Composition of MRS media

Table 1: Composition of MRS media

Ingredients	g/L
Protease	10.00
Beef extract	10.00
Yeast extract	5.00
Dextrose	20.00
Polysorbate 80	1.00
Ammonium citrate	2.00
Sodium citrate	5.00
Magnesium sulphate	0.1
Manganese sulphate	0.05
Dipotassium phosphate	2.00
pH(at 25^0C)	6.5 to 6.7

Laboratory preparation and set up of sorghum fermentation: The Sorghum was first cleaned by winnowing to remove chaffs and other light contaminants. It is then poured in a bowl of water so that the bad seed can float and be skimmed off. Then, it is washed by sterile distilled water 2 to 3 times. Then this sorghum grains were mixed with sterile distilled water in ratio of 1:2 (dry w/v). Then, this mixture was incubated at 37 ºC temperature for seven days in sterile utensils generally covered by gauze. During fermentation, samples were aseptically withdrawn for its physicochemical and microbiological analysis. Then isolation of bacteria or microbes on preferable selective media was done from fermented batter followed by characterization of these bacteria. Isolated bacteria/microbes were preserved though routine culture maintenance techniques in multiple quantity for further and subsequent use.

Isolation and Identification of microbial flora: A total 6 fermentation sets were subjected for study and analysis. Enumeration and isolation of microbial flora from fermentation for Lactic acid bacteria was done using standard microbiological techniques like serial dilution and pour plate technique. MRS medium was used under microaerophillic condition for 24h. From the fermented medium flask, 0.1mL of sample was taken on the solid MRS medium with 2 % (w/v) agar. It was spread with sterile glass rod until the suspension was properly adsorbed throughout on agar surface. Then, it was poured with 4-5mL liquefied agar with concentration of 1.5 % (w/v). The plates were incubated at 37 ºC for 48h. After incubation, isolated colonies were picked up from each plate and transferred to MRS broth and incubated at 37ºC for 48h for enrichment of isolates. The isolates were identified using standard morphological and cultural characteristics. All the isolates were initially tasted for Gram's reaction, Catalase test and production of acid from glucose (Hamed *et al*., 1992).

Isolation and biochemical characterization: The isolation was made by inoculating the culture from fermentation set on solid MRS agar plate. The well isolated and morphologically distinct colonies from the plates were selected and stock cultures were prepared for further analysis. All these isolates were further characterized in accordance with the standard tests prescribed in Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994).

RESULTS & DISCUSSIONS

The primary aim of this study was to check the effect of various utensils on steeped sorghum grain fermentation to prepare fermented batter. In the present study, 500 g of sorghum grains were used for fermentation in sterile water in various containers for about 144 h and among these samples examined with the help standard morphological, cultural, biochemical test and physical parameter the different colony counts were found.

The pH value of fermented batter decreases in all the utensils nearly to pH 3.5 during sorghum fermentation, this result correlated with (Sulieman, 2009) stated that during fermentation the pH dropped.

The pH values of fermented batter obtained are shown above table 2. In all utensils such as glass, plastic, steel, brass, clay and aluminum respectively observed at zero time to 48h nearly pH 6, 5.5 and 5. And after 72 h to 120 h, slightly decreases the pH nearly to 4.5 and 4.0 and in the 144 h again, decreases in all the utensils nearly to pH 3.5.

Mohammed *et al*., (1991) found some lactic acid bacterial species in sorghum fermentation as *Pediococcus pentosaceus*, *Lactobacillus confusus*, *Lactobacillus brevis*, *Lactobacillus sp*., *Erwinia ananas*, *Klebsiella pneumoniae* and *Enterobacter cloacae*. In sorghum, we too, found lactic acid bacterial species such as *Lactobacillus sp*., *Pediococcus sp*. and *Streptococcus sp.* They got dominant species *P. pentotaceus* and we observed *Streptococcus* species as dominant.

The physical parameter such as color, texture, viscosity, gas production, odor and consistency were studied and the results were obtained and summarized in the above table 3. The color of the fermented batter at 24 h was found to be yellow, no change after 48 h and slightly blackish after 72 h in glass utensil and plastic utensil was observed. Texture of the fermented batter was rough in all utensils studied. Viscosity was observed only in steel and brass after 72 h. Gas production was also observed in the glass and steel after 72 h. Sour odor was observed in all the fermented batter kept in various utensils.

Table 2: The pH value of fermented batter

Types of utensil	Zero time	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
Glass	5.0	5.0	5.5	5.0	4.0	4.0	3.5
Plastic	6.0	6.0	6.0	5.5	5.0	4.0	4.0
Steel	6.0	5.5	6.0	5.5	5.0	4.5	3.5
Brass	6.0	5.5	6.0	6.0	5.5	4.5	3.5
Clay	5.5	6.0	6.0	5.0	5.0	5.0	4.0
Aluminum	5.5	6.0	6.0	5.5	5.5	4.5	3.5

Table 3 : Physical Parameters

	Glass			Plastic			Steel			Brass			Clay			Aluminum		
Time	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Colour	Y	NC	SB	Y	NC	SB	Y	NC	WB	SB	SB	SB	Y	Y	SB	Y	SB	SB
Texture	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Viscosity	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-
Gas Production	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Odor	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Consistency	W	W	W	W	W	W	W	W	W	W	W	W	D	D	D	W	W	D

Abbreviation: Y= Yellowish, NC=N Change, SB=Slightly Blackish, WB=whitish Blackish, R=Rough, S=Sour, W= watery, D=Drain

Table 4: Numbers of colonies observed on MRS agar media

Utensils	Glass			Plastic			Steel			Brass			Clay			Aluminum		
	Incubation time in hrs																	
Fermentation time in hours	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
	No. of colony CFU/ml																	
Zero time	0.97 X 10^9	1.15 X 10^9	1.52 X 10^9	0.60 X 10^9	0.65 X 10^9	0.70 X 10^9	0.02 X 10^9	0.04 X 10^9	0.04 X 10^9	0.07 X 10^9	0.16 X 10^9	0.29 X 10^9	0.02 X 10^9	0.08 X 10^9	0.14 X 10^9	0.26 X 10^9	0.33 X 10^9	0.40 X 10^9
24 hrs	1.80 X 10^9	1.93 X 10^9	1.98 X 10^9	0.78 X 10^9	1.75 X 10^9	1.77 X 10^9	0.11 X 10^9	0.17 X 10^9	0.21 X 10^9	0.12 X 10^9	0.61 X 10^9	0.82 X 10^9	0.12 X 10^9	0.19 X 10^9	0.19 X 10^9	0.82 X 10^9	0.84 X 10^9	0.88 X 10^9
48 hrs	2.11 X 10^9	2.30 X 10^9	2.50 X 10^9	3.87 X 10^9	3.92 X 10^9	4.02 X 10^9	5.20 X 10^9	5.43 X 10^9	5.65 X 10^9	0.80 X 10^9	2.69 X 10^9	3.49 X 10^9	0.18 X 10^9	0.33 X 10^9	0.35 X 10^9	4.20 X 10^9	4.20 X 10^9	4.20 X 10^9
72 hrs	2.85 X 10^9	2.64 X 10^9	2.44 X 10^9	0.34 X 10^9	0.30 X 10^9	0.28 X 10^9	0.26 X 10^9	0.42 X 10^9	0.53 X 10^9	0.90 X 10^9	3.60 X 10^9	4.68 X 10^9	0.78 X 10^9	0.82 X 10^9	0.85 X 10^9	3.54 X 10^9	3.64 X 10^9	3.72 X 10^9
96 hrs	3.12 X 10^9	3.24 X 10^9	3.32 X 10^9	1.55 X 10^9	1.25 X 10^9	1.85 X 10^9	0.02 X 10^9	0.36 X 10^9	0.82 X 10^9	0.71 X 10^9	1.40 X 10^9	1.58 X 10^9	4.46 X 10^9	5.89 X 10^9	6.24 X 10^9	7.68 X 10^9	7.74 X 10^9	7.82 X 10^9
120 hrs	3.48 X 10^9	3.54 X 10^9	3.62 X 10^9	1.13 X 10^9	2.66 X 10^9	2.82 X 10^9	2.28 X 10^9	2.32 X 10^9	2.48 X 10^9	2.76 X 10^9	2.96 X 10^9	3.02 X 10^9	2.41 X 10^9	2.42 X 10^9	2.42 X 10^9	8.56 X 10^9	8.72 X 10^9	8.72 X 10^9
144 hrs	4.80 X 10^9	4.89 X 10^9	4.92 X 10^9	2.46 X 10^9	4.12 X 10^9	4.24 X 10^9	2.13 X 10^9	2.55 X 10^9	2.78 X 10^9	2.10 X 10^9	4.32 X 10^9	4.89 X 10^9	11.44 X 10^9	11.96 X 10^9	11.96 X 10^9	2.33 X 10^9	2.33 X 10^9	2.33 X 10^9

Table 5: Morphological, Culture and Biochemical Chacteristics

No.of Isolates	Test		S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
Moorpogical Characteristics	**Cell Shape**		Short rod	Cocci in buches	Cocci in buches	Short rod	Cocci in buches	Short rod	Cocci in buches	Short rod	Cocci in buches	Cocci in buches
	Gram Reaction		+	+	+	+	+	-	+	-	+	+
	Motility		NM	NM	NM	NM	NM	NM	NM	NM	NM	NM
	Endospore Formation		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Culture Characteristics	**Colour of colony**		White	White	White	White	White	White	White	Pale Yellow	White	Pale Yellow
	Colony Shape		Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
	Margin		Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
	Elevation		Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex	Convex
	Density		Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Biochemical Characteristics	**Catalase**		-	-	-	-	-	-	-	-	-	-
	oxidase		-	-	-	-	-	-	-	-	-	-
Sugur Fermentation	**Glucose**	**A**	+	-	+	-	+	+	+	+	+	+
		G	+	+	+	-	+	+	-	-	-	-
	Mannitol	**A**	-	-	-	-	-	+	+	-	-	-
		G	-	+	-	+	-	+	+	-	+	-
	Sucrose	**A**	-	-	-	-	+	+	-	+	-	-
		G	-	-	+	-	-	+	-	-	+	-
	Lactose	**A**	-	-	-	-	-	+	+	+	-	-
		G	+	+	-	+	+	+	+	+	+	+
	Fuctose	**A**	+	+	+	-	+	+	+	+	+	+
		G	-	-	-	+	+	+	-	+	+	-
	Probable Microorganism		*Lacto-bacillus* spp.	*Strepto-coccus* spp.	*Strepto-coccus* spp.	*Lacto-bacillus* spp.	*Strepto-coccus* spp.	*Lacto-bacillus* spp.	*Strepto-coccus* spp.	*Lacto-bacillus* spp.	*Strepto-coccus* spp.	*Pedio coccus* spp.

Where NM= Non motile, NS= Non Sporulating, A =Acid, G= Gas, (-) Negative, (+)= Positive

The numbers of colonies observed on MRS agar media are showed in above data table 4. The colonies were observed after 24 h, 48h and 72 h on the plates incubated. The colonies on plate incubated at 72 h. Vigorous growth and colonies of bacteria were observed on earthen pot. These findings suggested that the earthen pot is the favorable for the growth of bacteria in the fermented batter.

Morphological, cultural and biochemical characteristics were shown in the above table 5. Sorghum fermentation was carried out for the preparation of fermented batter in different utensils like glass, plastic, steel, brass, clay and aluminum respectively. Then, these physical parameters, morphological, cultural and biochemical characteristics of different microorganism were observed. The probable microorganisms were *Lactobacillus sp., Streptococcus sp.* and *Pediococcus sp.* Then, all these observations and results were subjected to conclusion for the preparation of fermented batter prepared in clay utensil.

The main intension for using clay utensil was to maintain the proper temperature and also inert nature. This observation has also been made by other studies (Carter and Tyrrel, 2004). Clay storage containers maintained water temperature at about 4.5°C lower than the mean source water temperature as a result of aeration through the porous material and were preferred over other container types for this reason. Because adequate aeration is possible, draining of excess water is most suitable for fermentation and species of microbes as a substratum an effective fermentation. Khan and Banerjee (2020) suggested that food made with clay utensil was very healthy and tasty and through clay pottery new employment opportunity can be generated. Now days, global focus is on natural resources, so clay utensils waste management will not take any space for waste. From this conclusion clay utensil is superior to other than utensils such as aluminum, steel, glass, brass and plastic.

CONCLUSIONS

The effect of utensils on the growth of Lactic acid bacteria showed an ecological relationship. As the time of fermentation increases, the pH of Fermentation batter lowers due to the acidic fermentation. Lactic acid bacteria were found in fermentation sets were *Streptococcus sp*., *Lactobacillus sp*., *Pediococcus sp*. Out of these *Streptococcus* species were dominant. The Lactic acid bacterial population dynamics showed higher in utensil, the following order Clay utensil > Aluminum utensil > Steel utensil > Glass utensil > Brass utensil > Plastic utensil, thus, clearly indicating that in household sorghum fermentation clay utensil is better than other utensils.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Abah, CR, Ishiwu CN, Obiegbuna JE and Oladejo AA (2020) Sorghum Grains: Nutritional Composition, Functional Properties and Its Food Applications. *European Journal of Nutrition & Food Safety*, 12(5): 101-111.

Butti P and More DR (2016) Studies on physico-chemical analysis of Sorghum varieties. *Int. J. Agri. Sci. & Res*., 6(1): 87-92.

Carter RC and Tyrrel SF (2004) Water quality deterioration: a study of household drinking water quality in rural Honduras. *International journal of environmental health research*, 14(4): 273-283.

Day C and Morawicki R (2018) Effects of Fermentation by Yeast and Amylolytic Lactic Acid Bacteria on Grain Sorghum Protein Content and Digestibility. *Journal of Food Quality*, 1-8.

De Man JC, Rogosa M and Sharpe ME (2008) A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology*, 23(1): 130-135.

Hamed SH, Boecker G, Vogel RFand Hammes WP (1992). Microbiological analysis of fermented sorghum dough for kisra production, J. Appl. Microbiol.(37):728-731.

Holt JG, Krieg N, Sneath PHA, Staley JT, and Williams ST (1994) Bergey's Manual of Determinative Bacteriology, 9th ed. Baltimore (MD): Williams & Wilkins.

Khan D and Banerjee S (2020) Revitalizing ancient Indian clay utensils and its impact on health. *International Journal of All Research Education and Scientific Methods (IJARESM)*. 8(7): 357-360.

Kunene NF, Geornaras I, Holy AV and Hastings JW (2000) Characterization and Determination of Origin of Lactic Acid Bacteria from a Sorghum-Based Fermented Weaning Food by Analysis of Soluble Proteins and Amplified Fragment Length Polymorphism Fingerprinting. *Journal of Applied and Environmental Microbiology*, 66(3): 1084–1092.

Mohammed SI, Steenson LR and Kirleis AW (1991) Isolation and characterization of microorganisms associated with the traditional sorghum fermentation for production of

sudanese kisra. *Journal of Applied and Environmental Microbiology*, 57(9): 2529-2533.

Ojokoh AO, Alade PT, Ozabor and Fadahunsi IF (2020)Effect of fermentation on sorghum and cowpea flour blends. *J. Agric. Biotech. Sustain. Dev.* 12(2): 39-49.

Prabhakar B and More DR (2016) Studies on physico-chemical analysis of sorghum varieties. *International Journal of Agricultural Science and Research*, 6(1): 87-92.

Sulieman AME (2009) Effect of fermentation on the nutritional and microbiological quality of dough of different sorghum varieties. *J. Sc. Tech.*, 10(3): 101-119.

2023 | Published by IJLSCI